1/15/2002

Docket No: AdVec10IA-C1
Applicant: Graham, et al.
Filed: 10/16/2001
Serial No: 09/978,464

RECEIVED
FEB 15 2002
TECHNOLOGY CENTER 2800

For Submission to the USPTO:

1. Response to Notice to File Missing Parts (trans.)
2. executed declaration & power of attorney
3. basic filing fee: $740.00
4. additional claims fee: $840.00
5. missing parts surcharge: $130.00
6. substitute drawings
7. abstract
8. sequence listing on disk
9. sequence listing statement

OIPE JC87
FEB 11 2002
PATENT & TRADEMARK OFFICE

Received from < 407 228 0329 > at 5/9/02 3:31:23 PM [Eastern Daylight Time]

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WASHINGTON, D.C. 20231
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| APPLICATION NUMBER | FILING/RECEIPT DATE | FIRST NAMED APPLICANT | ATTORNEY DOCKET NUMBER |
|---|---|---|---|
| 09/978,464 | 10/16/2001 | Frank L. Graham | ADVEC101A-C1 |

CONFIRMATION NO. 5099

29847
VAN DYKE & ASSOCIATES, P.A.
1630 HILLCREST STREET
ORLANDO, FL 32803

FORMALITIES LETTER

*OC000000007075530*

Date Mailed: 11/15/2001

## NOTICE TO FILE MISSING PARTS OF NONPROVISIONAL APPLICATION

**FILED UNDER 37 CFR 1.53(b)**

***Filing Date Granted***

An application number and filing date have been accorded to this application. The item(s) indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The statutory basic filing fee is missing.
  *Applicant must submit $ 740 to complete the basic filing fee for a non-small entity. If appropriate, applicant may make a written assertion of entitlement to small entity status and pay the small entity filing fee (37 CFR 1.27).*
- Total additional claim fee(s) for this application is $840.
  - **$252** for **14** total claims over 20.
  - **$588** for 7 independent claims over 3 .
- The oath or declaration is unsigned.
- To avoid abandonment, a late filing fee or oath or declaration surcharge as set forth in 37 CFR 1.16(f) of $130 for a non-small entity, must be submitted with the missing items identified in this letter.
- **The balance due by applicant is $ 1710.**

The application is informal since it does not comply with the regulations for the reason(s) indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- Substitute drawings in compliance with 37 CFR 1.84 because:
  - drawing sheets do not have the appropriate margin(s) (see 37 CFR 1.84(g)). Each sheet must include a top margin of at least 2.5 cm. (1 inch), a left side margin of at least 2.5 cm. (1 inch), a right side margin of at least 1.5 cm. ( 5/8 inch), and a bottom margin of at least 1.0 cm. (3/8 inch).
- An abstract was not provided for this application. An abstract of the technical disclosure is required under 37 CFR 1.72(b).

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- The paper or compact disc copy of the "Sequence Listing" is not the same as the computer-readable form of the "Sequence Listing" as required by 37 CFR 1.821(e). Applicant must provide a substitute paper or compact disc copy of the "Sequence Listing", as well as an amendment directing its entry into the application OR a substitute computer readable form (CRF) copy of the "Sequence Listing". These two items must be the same. Applicant must also provide a statement that the content of the sequence listing information recorded in computer readable form is identical to the written (on paper or compact disc) sequence listing and, where applicable, includes no new matter, as required by 37 CFR 1.821(e), 1.821(f), 1.821(g), 1.825(b), or 1.825(d). If the effective filing date is on or after September 8, 2000, see the final rulemaking notice published in the Federal Register at 65 FR 54604 (September 8, 2000) and 1238 OG 145 (September 19, 2000).

**For questions regarding compliance to these requirements, please contact:**

- **For Rules Interpretation, call (703) 308-4216**
- **To Purchase PatentIn Software, call (703) 306-2600**
- **For PatentIn Software Program Help, call (703) 306-4119 or e-mail at patin21help@uspto.gov or patin3help@uspto.gov**

---

***A copy of this notice MUST be returned with the reply.***

Customer Service Center
Initial Patent Examination Division (703) 308-1202
PART 2 - COPY TO BE RETURNED WITH RESPONSE

*1630 Hillcrest Street*
*Orlando, Florida 32803*
*USA*

**Van Dyke & Associates, PA**
INTELLECTUAL PROPERTY LAW

*Phone: (407) 228-0328*
*Fax: (407) 228-0329*
*info@patentinternational.com*
*www.patentinternational.com*

*FACSIMILE COVER SHEET*

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| | | |
|---|---|---|
| TO | : | USPTO |
| FAX No. | : | 703-746-9195 |
| No of PAGES | : | 45 (including cover sheet) |
| FROM | : | Van Dyke & Associates, P.A. |
| DATE | : | May 9, 2002 |
| RE: | : | Serial No: 09/978,464<br>Our Docket No: AdVcc101A-C1 |

**VIA FACSIMILE ONLY**

To Whom It May Concern:

Please see the attached as requested in the "Request for Substitute Papers" mailed on April 25, 2002. The Request has been signed and dated clarifying that this facsimile submission is complete and accurate as originally filed.

Applicant also provides herewith a copy of an executed Revocation & New Power of Attorney for this case, which is the last page of this facsimile.

If you have any questions regarding this correspondence, please feel free to contact me.

Regards,

Timothy H. Van Dyke, Reg. No. 43218
Van Dyke & Associates, P.A.
TVD/mkk
Attachments

*If you do not receive all pages or if any portion of this transmission is not legible, call the sender at (407) 228-0328.*

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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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| Application Number | Filing Date | First Named Applicant | Atty. Docket No. |
|---|---|---|---|
| 09/978,464 | 10/16/2001 | Graham, Frank | ADVEC101A-C1 |

29847
VAN DYKE & ASSOCIATES, P.A.
1630 HILLCREST STREET
ORLANDO FL 32803

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APR 29 2002
TECH CENTER

Title: Enhanced system for construction of adenovirus vectors

Date Mailed:04/25/2002

# Request for Substitute Papers

The papers filed on 02/11/02 (certificate of mailing dated 01/15/02 ) are no longer in condition to become part of the permanent records of the United States Patent and Trademark Office (USPTO) for this application (37 CFR 1.52(a)) due to the United States Postal Service sanitization process.

The USPTO requests that applicant provide a copy of the above-identified papers (except for any U.S. or foreign patent documents submitted with the above-identified papers) with a statement that such copy is a complete and accurate copy of the above-identified papers (signing and returning a copy of this notice will provide such a statement). The reply to this letter should be submitted to the USPTO by facsimile at the number indicated [redacted] 703-746-9195

Alternatively, the reply to this letter may be hand-carried to the Customer Service Window located in Room 1B03 of Crystal Plaza Building 2, Arlington, Virginia, 22202.

The USPTO **strongly** prefers that the reply to this letter be submitted by facsimile. However, if applicant cannot submit the reply to this letter by facsimile (or hand-delivery), the reply may be mailed to: Box Duplicate OIPE, U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202-2327.

This letter is not a notice under 37 CFR 1.251. However, failure to timely replay to this notice within two (2) weeks of the date of receipt of this letter may result in the USPTO issuing a notice under 37 CFR 1.251. **A copy of this notice should be included with the reply.**

---

The enclosed papers are a complete and accurate copy of the above-identified papers.

Name: Timothy A. Van Dyke Registration No.: 43,218

Signature: [signature] Date: 5-9-02

CTS 4/29/02
Date received
Docketed 4/30/02 By
Timely response due 5/9/02
Last date to file response 5/9/02



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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | |
|---|---|
| Applicant(s): Graham *et al.*<br>Application No.: 09/978,464<br>Filed: 10/16/2001<br>Title: Enhanced System for Construction of Adenovirus Vectors<br>Attorney Docket No.: ADVEC101A-C1 | Group Art Unit: 1636 |

**RESPONSE TO NOTICE TO FILE MISSING PARTS OF APPLICATION**

Assistant Commissioner for Patents
Washington, D.C. 20231
Attention: Box Missing Parts

Sir: This is in response to a Notice to File Missing Parts of Application under 37 CFR 1.53(b). Enclosed is a copy of said Notice and the following documents and fees to complete the filing requirements of the above-identified application.

(X) Copy of the executed Declaration and Power of Attorney from parent application serial no. 09/415,899.

(X) Statutory basic filing fee $740.00. (X) Utility ( ) Design

(X) Additional claim fees of $840.00 ($252.00 for 14 total claims over 20 and $588.00 for 7 independent claims over 3).

(X) Missing Parts Surcharge of $130.00.

(X) Substitute drawings in compliance with 37 C.F. R. 1.84.

(X) Abstract as required under 37 C.F.R. 1.72(b). This abstract was inadvertently omitted. The abstract, as provided herein, presents no new matter, and the 10/16/2001 filing date should stand as the filing date. Accordingly, it is requested under 37 USC 1.115 that the abstract, as provided herein, be added as page 99 to the application.

(X) Sequence Listing on disk.

(X) Sequence Listing Statement.

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope with sufficient postage addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Date of Deposit: 1/15/2002

Typed Name: Timothy H. Van Dyke

Signature:

Respectfully submitted,

By
Timothy H. Van Dyke, Reg. No. 43218

Date: 1/15/2002

Customer No: 29847
Van Dyke & Associates, P.A.
1630 Hillcrest Street
Orlando, FL 32803
Phone: 407-228-0328; Fax: 407-228-0329

Received from < 407 228 0329 > at 5/9/02 3:31:23 PM [Eastern Daylight Time]

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | |
|---|---|
| Applicant(s): Graham *et al.* | |
| Application No.: 09/978,464 | |
| Filed: 10/16/2001 | Group Art Unit: 1636 |
| Title: Enhanced System for Construction of Adenovirus Vectors | |
| Attorney Docket No.: ADVEC101A-C1 | |

**SEQUENCE LISTING STATEMENT UNDER 37 CFR 1.8 21-825**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The information recorded in computer readable form (diskette attached) is identical to the written sequence listing for the captioned application as filed with the United States Patent and Trademark Office. Furthermore, the content of the sequence listing information recorded in computer readable form contains no new matter.

Respectfully submitted,

1-15-2002
Date

Timothy H. Van Dyke, Reg. No. 43218
Attorney for Applicants

Customer No: 29847

Van Dyke & Associates, P.A.
1630 Hillcrest Street
Orlando, Florida 32803
Phone: 407-228-0328
Fax: 407-228-0329

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## ABSTRACT OF THE DISCLOSURE

In the present invention, viruses, plasmids or both are constructed which contain viral DNA, at least one head-to-head ITR junction, and recombinase recognition sites positioned such that site-specific recombination between recombinase recognition sites in separate plasmids results in generation of infectious viral DNA at high-efficiency in cotransfected host cells that have been engineered to express a site-specific recombinase. Because of the high-efficiency and specificity of the Cre enzyme, suitably engineered plasmids can be readily recombined to produce infectious virus at high-efficiency in cotransfected 293 cells, without, at the same time, producing wild-type adenovirus, with the attendant problems for removal thereof. Use of recombinases besides Cre and recombinase recognition sites besides lox sites, and use of cells other than 293 cells are also disclosed and enabled, as are kits incorporating the site-specific vector system, as well as compositions and methods for using such compositions as vaccines or in gene therapeutic applications. Enhancement in the efficiency of site-specific recombination is provided by inclusion of a head-to-head ITR junction in each virus, plasmid, or other nucleic acid construct.

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Cotransfection of 293Cre cells with pBHG10lox and a "Lox" shuttle plasmid for generation of Ad expression vectors

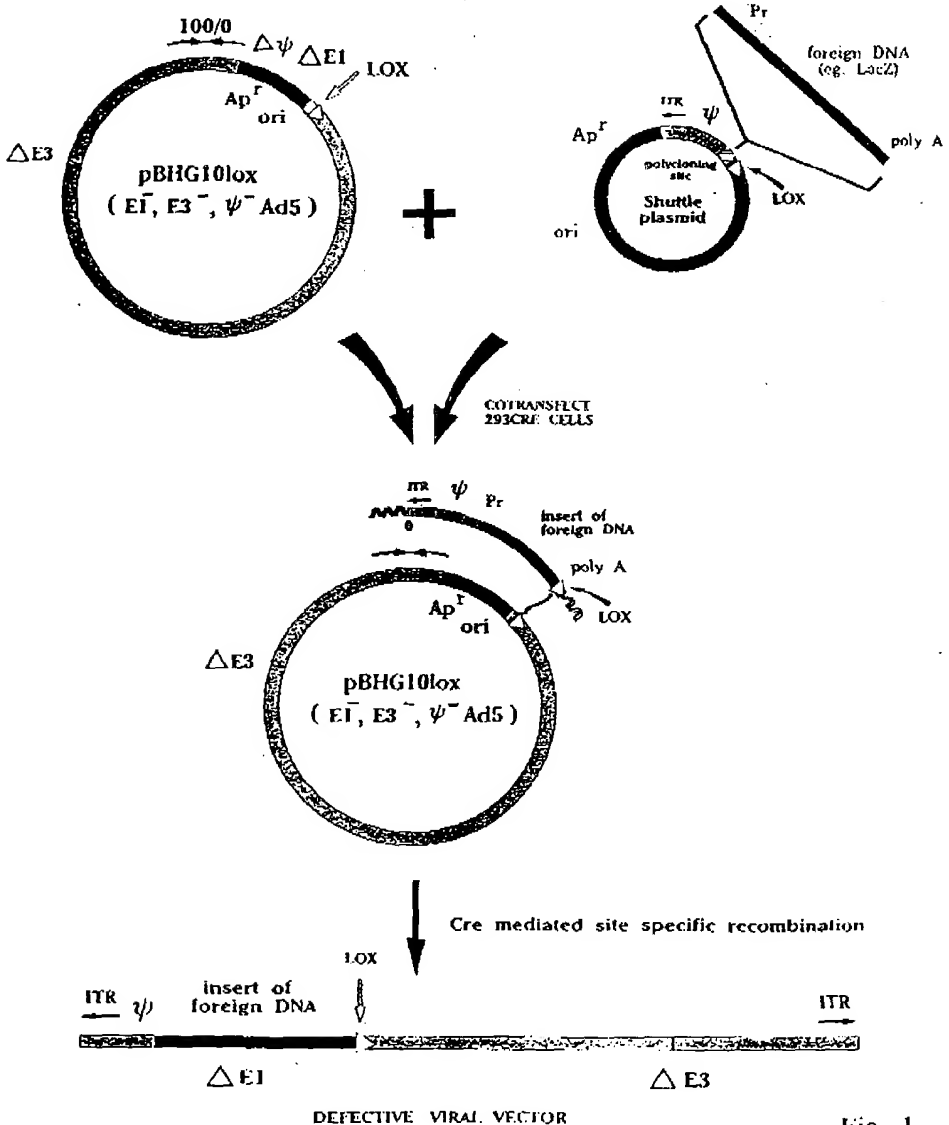
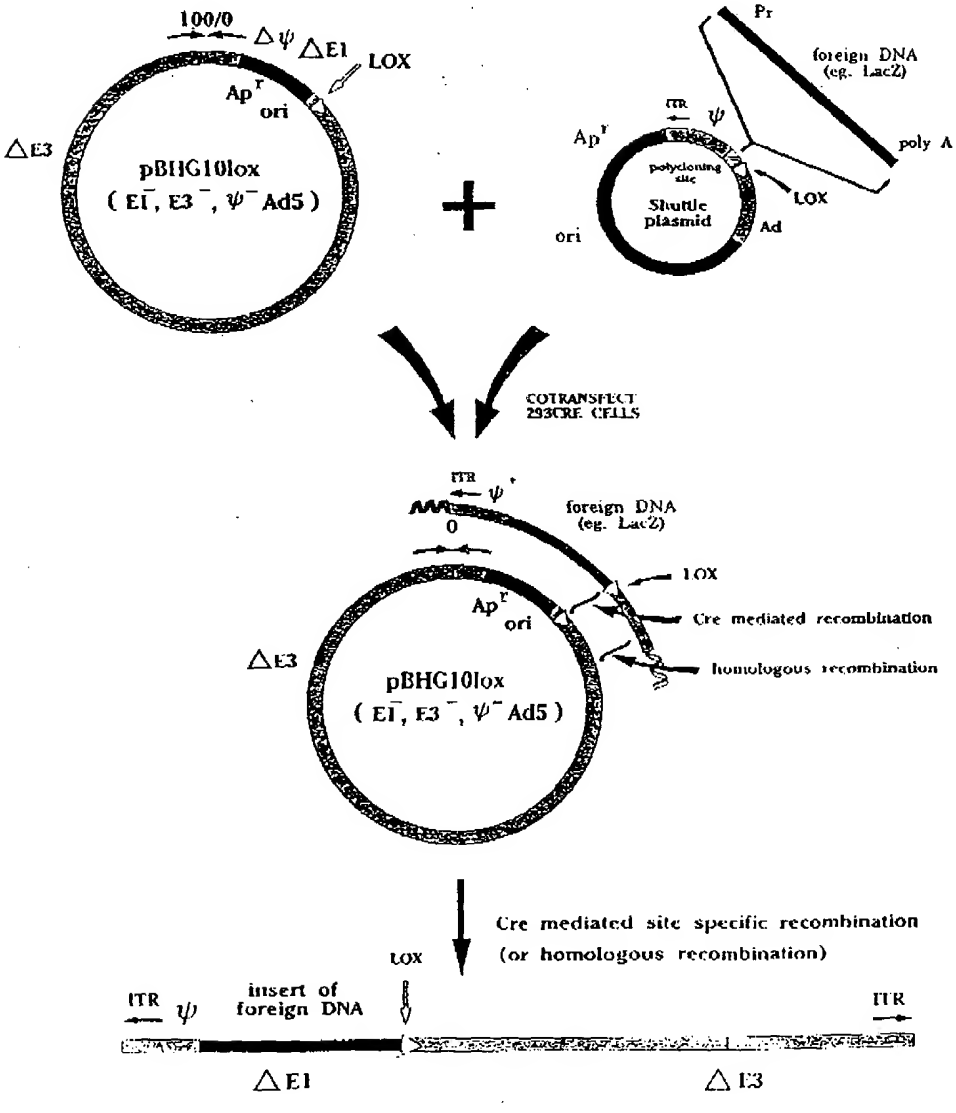



Fig. 1

**Cotransfection of 293Cre cells with pBHG10lox and a "lox" shuttle plasmid for generation of Ad expression vectors**




DEFECTIVE VIRAL VECTOR

Fig. 2A

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# CONSTRUCTION OF VARIOUS SHUTTLE PLASMIDS

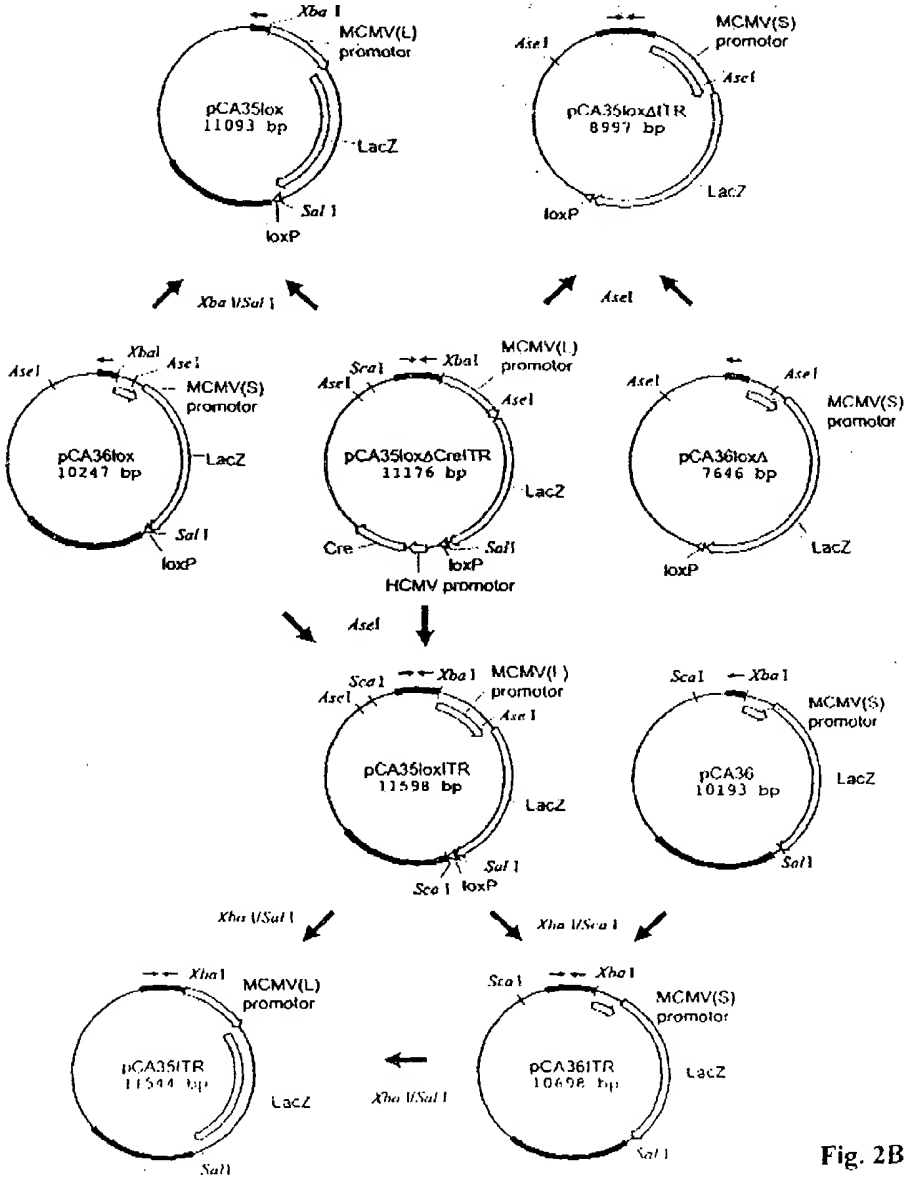
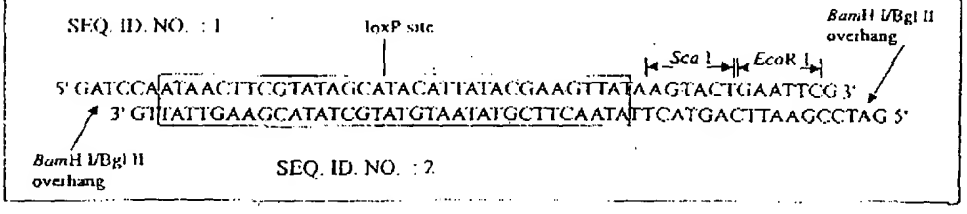


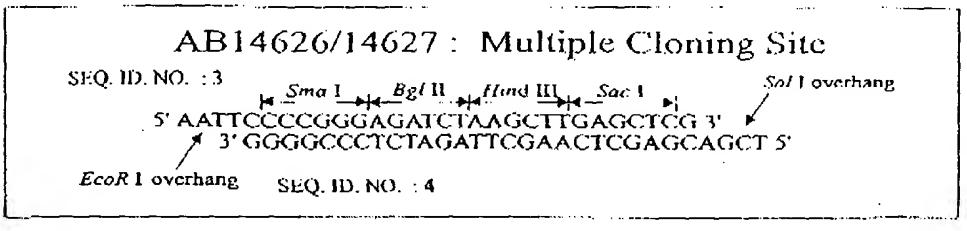

Fig. 2B

# OLIGONUCLEOTIDES USED IN CLONING

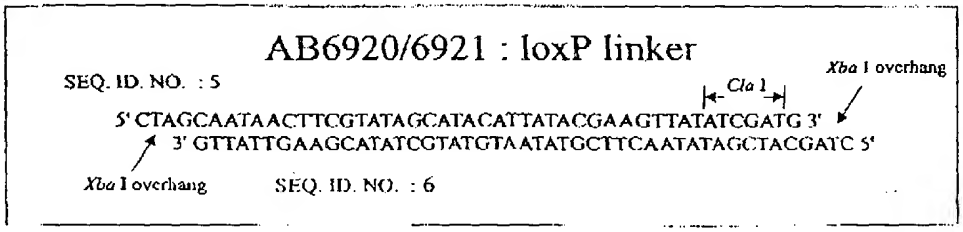
## AB3233/3234 : loxP linker




## AB14626/14627 : Multiple Cloning Site




## AB6920/6921 : loxP linker




## AB14680/14681 : loxP linker

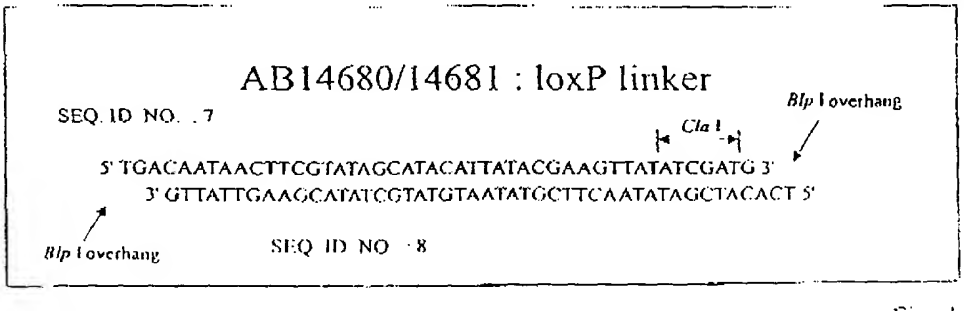



Fig. 3

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# CONSTRUCTION OF A CIRCULAR GENOMIC PLASMID FOR Ad VECTOR RESCUE USING THE Cre/ loxP SYSTEM

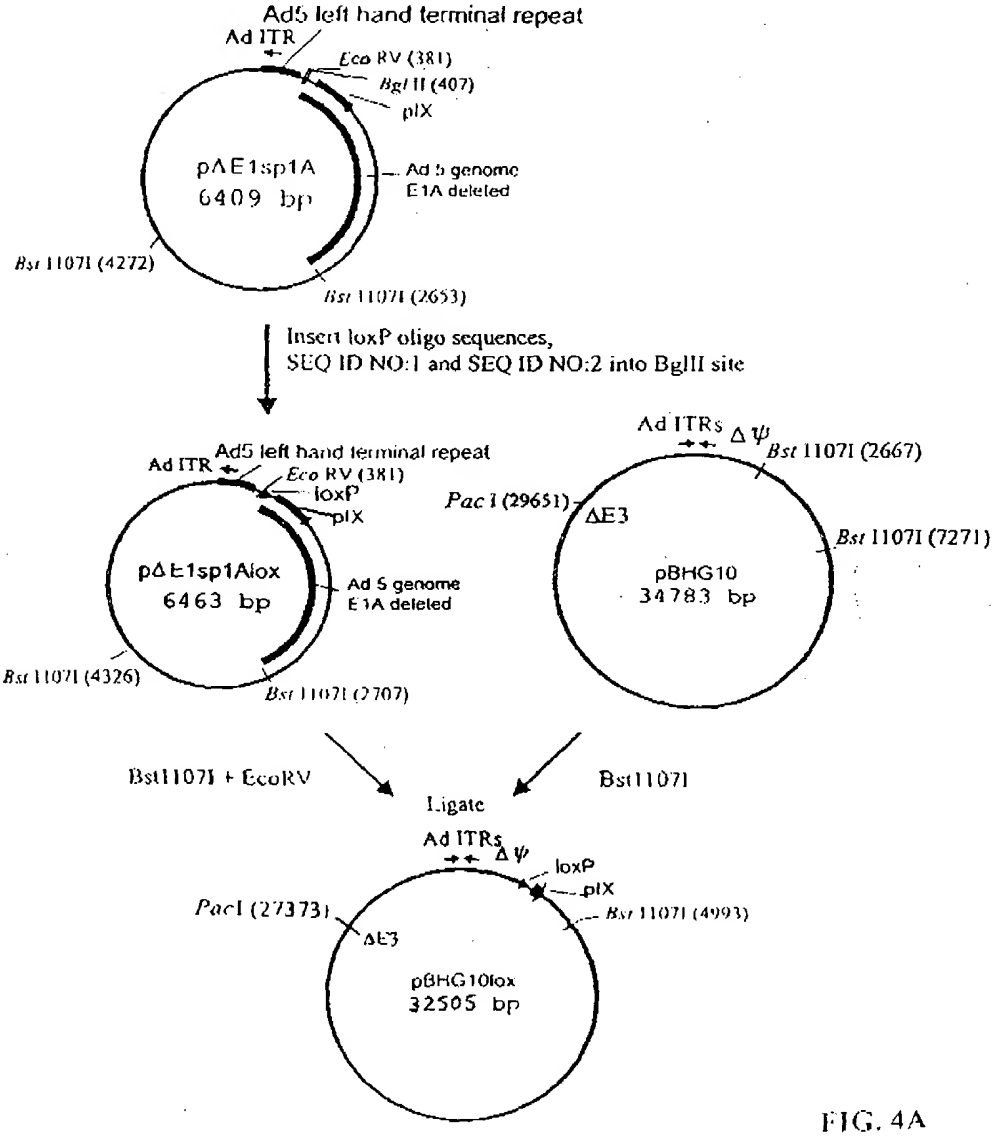


FIG. 4A

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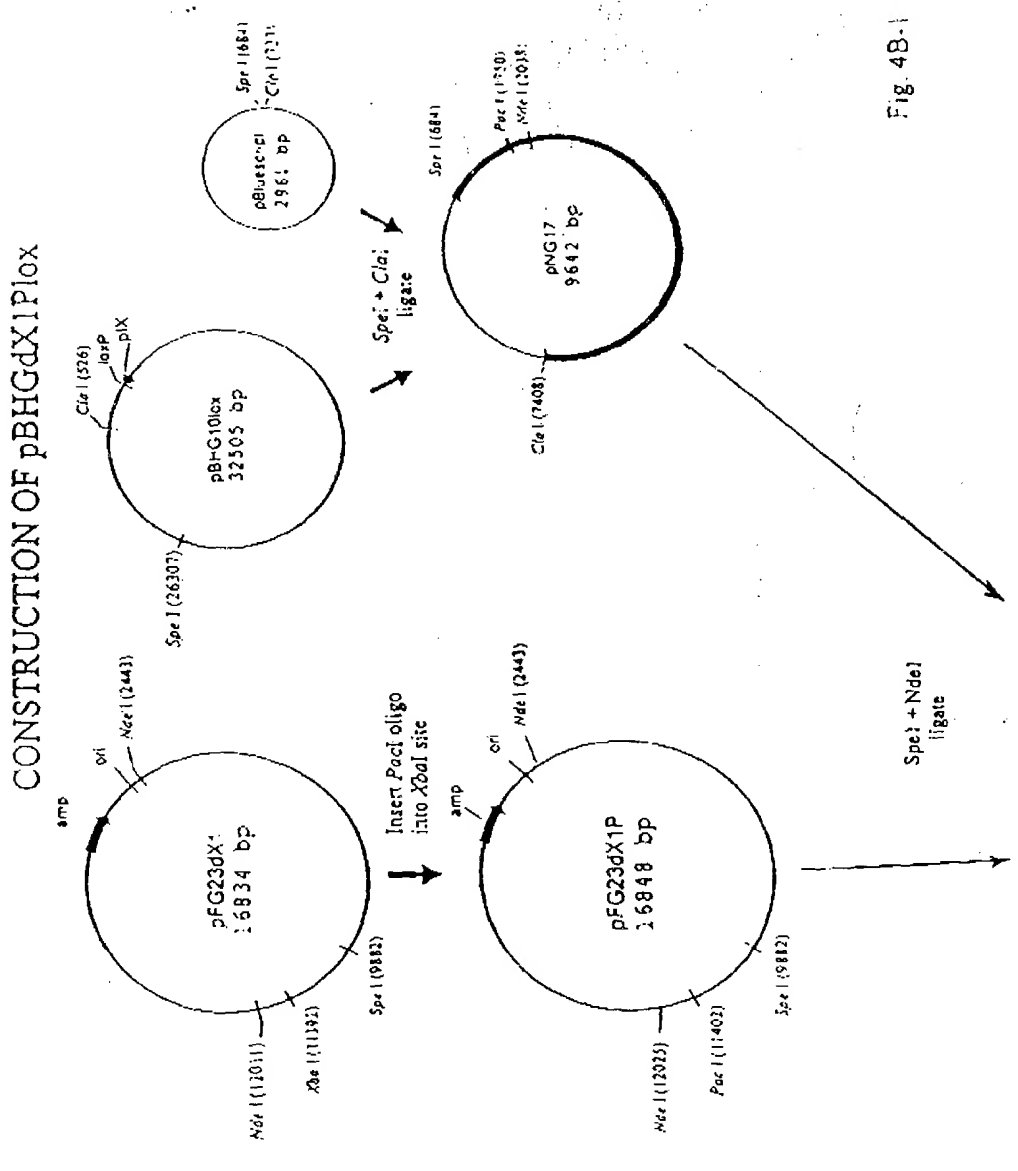
CONSTRUCTION OF pBHGdX1Plox
pBluescript
2961 bp
Spe I (6841)
Cla I (7211)
pBHG10lox
32505 bp
Cla I (526)
loxP
pIX
Spe I (26307)
Spe I + Cla I
ligate
pNG17
9642 bp
Spe I (6841)
Pac I (1210)
Nde I (3035)
Cla I (7408)
pFG23dX1
16834 bp
amp
ori
Nde I (2443)
Spe I (9812)
Xba I (11321)
Not I (12011)
Insert Pac I oligo
into Xba I site
pFG23dX1P
16848 bp
amp
ori
Nde I (2443)
Spe I (9812)
Pac I (11402)
Not I (12025)
Spe I + Nde I
ligate

Fig. 48-1

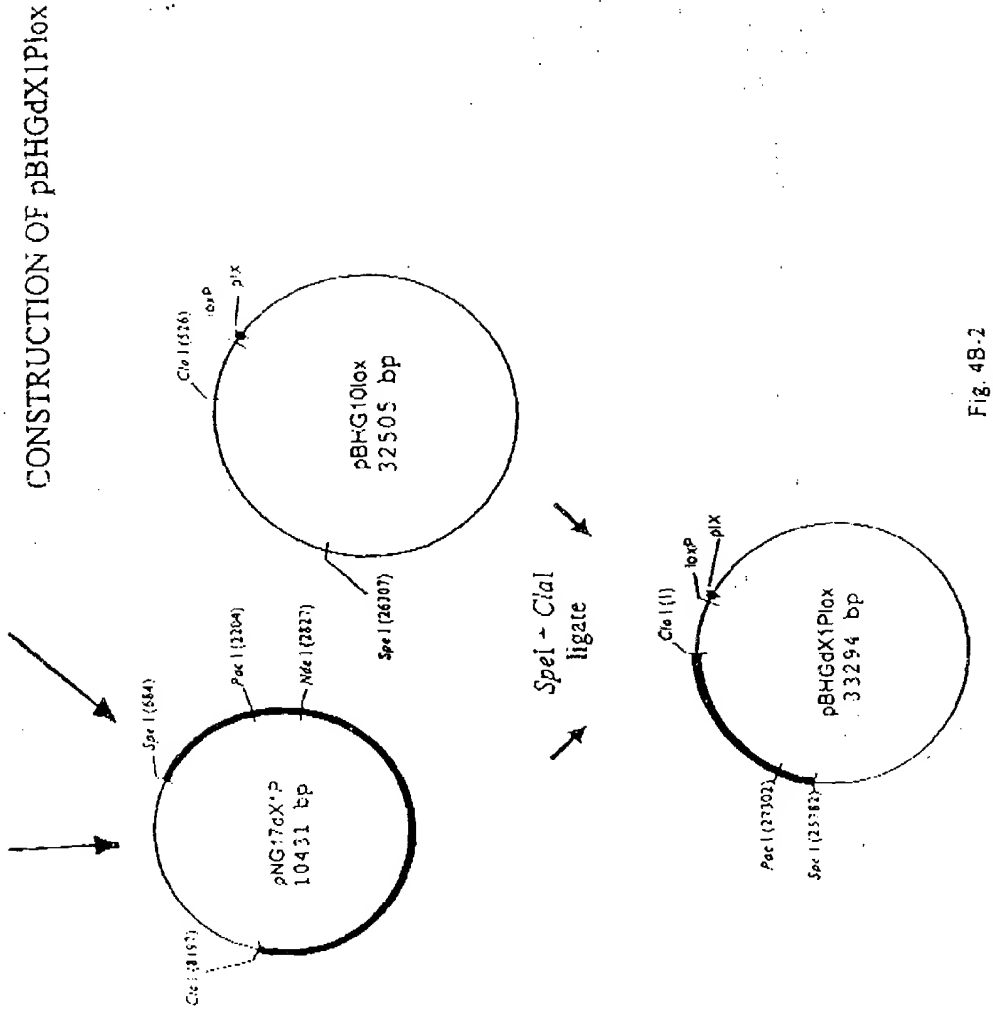


Fig. 48-2

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# CONSTRUCTION OF pBHGE3lox

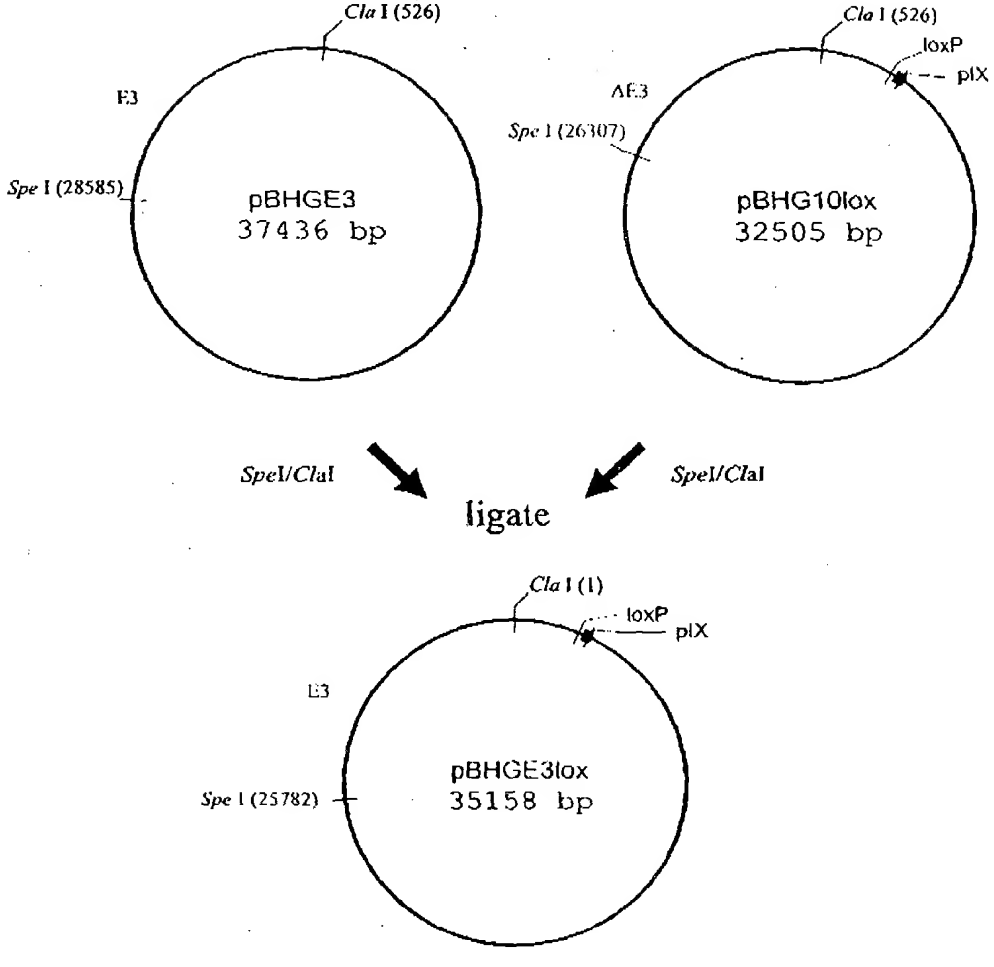


Fig. 4C

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# CONSTRUCTION OF Ad GENOMIC PLASMIDS ENCODING CRE

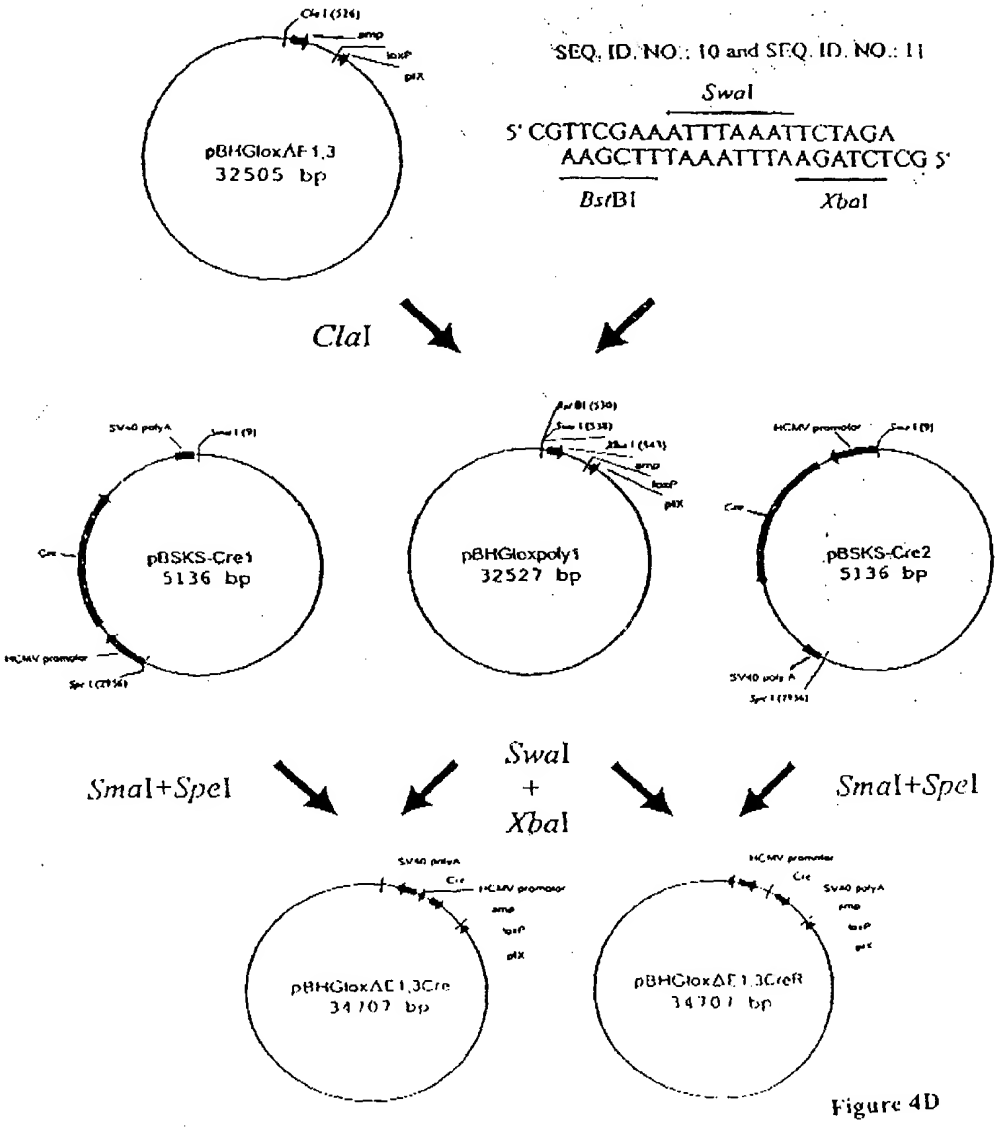


Figure 4D

# CONSTRUCTION OF pΔE1SP1A & pΔE1SP1B loxP PLASMIDS FOR RESCUE OF FOREIGN DNA

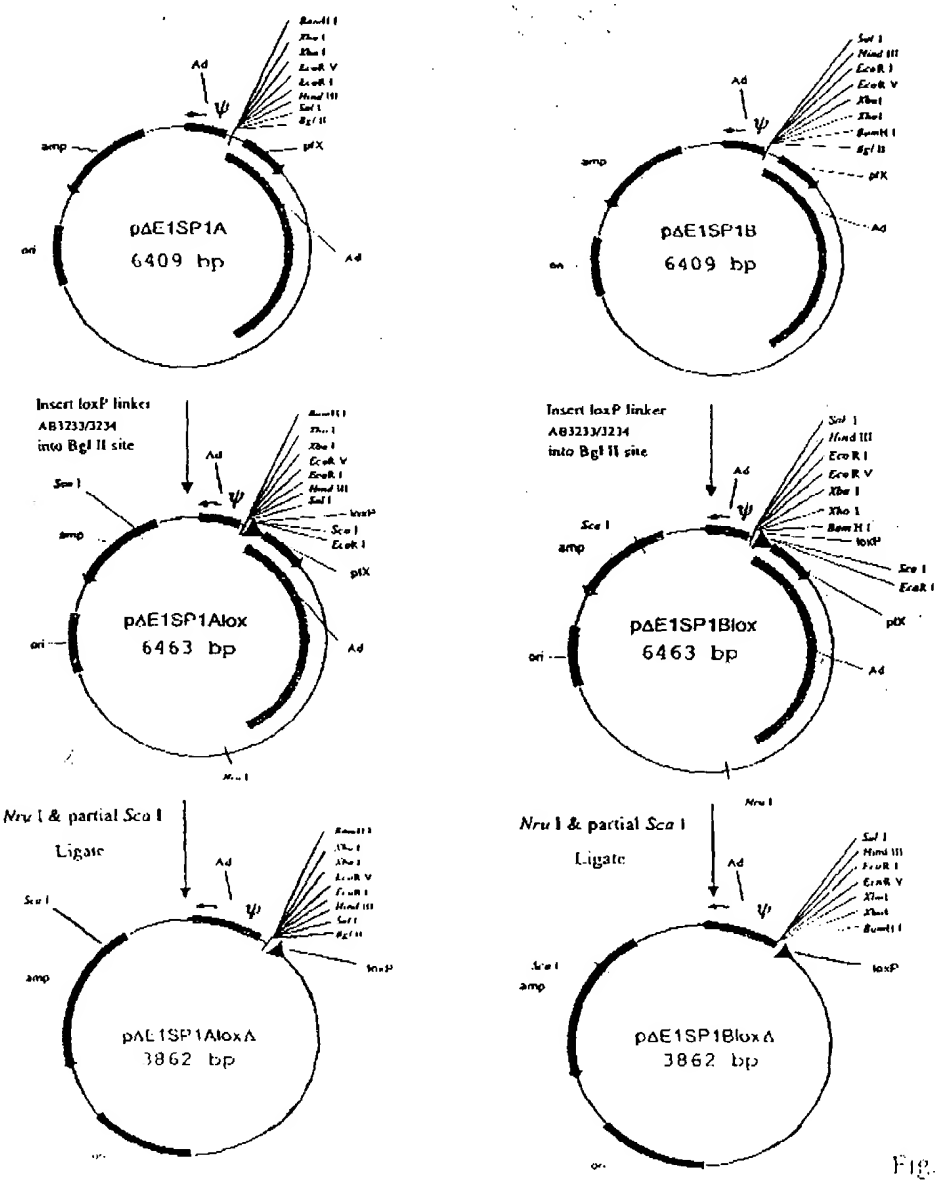


Fig. 5A

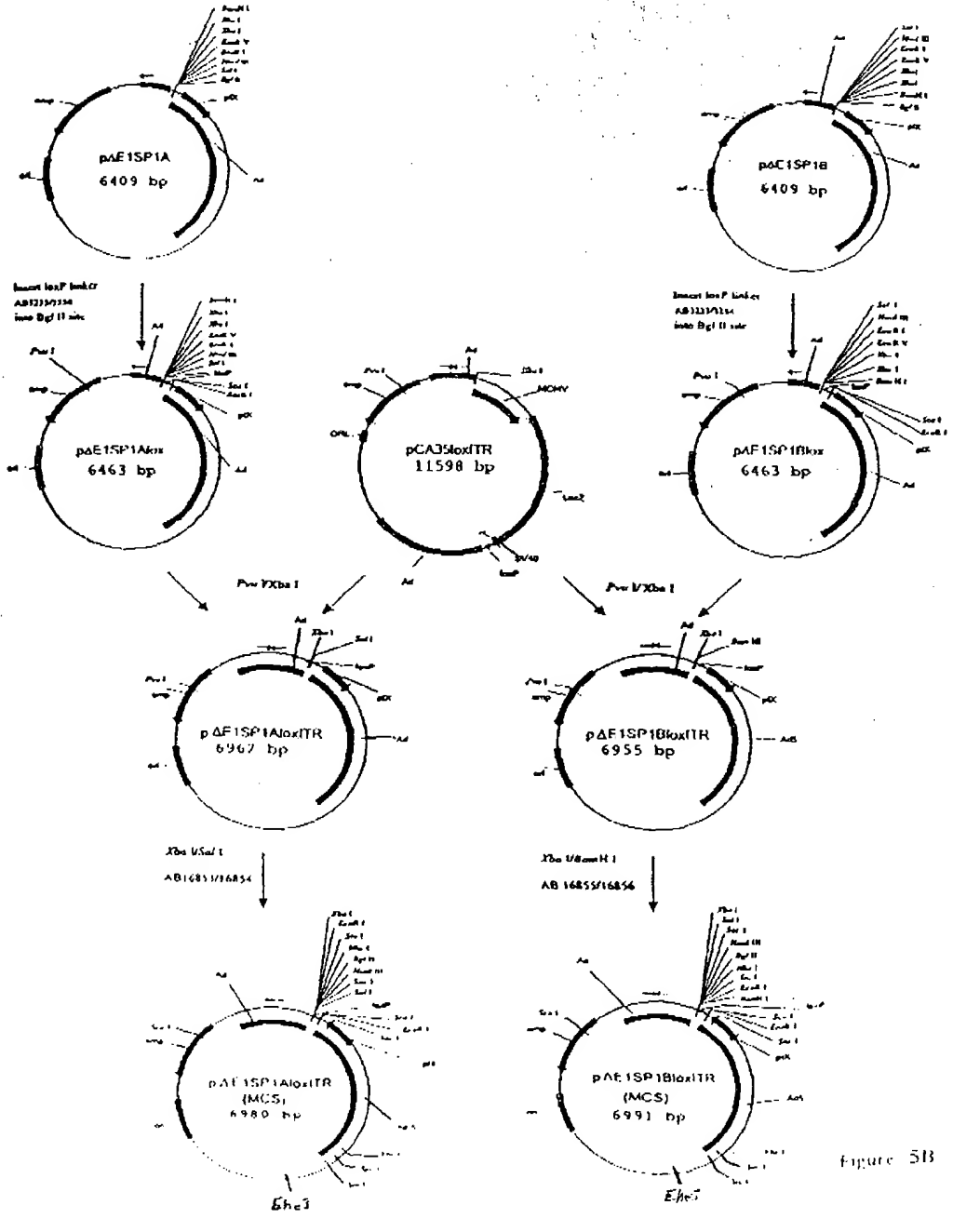


Figure 5B

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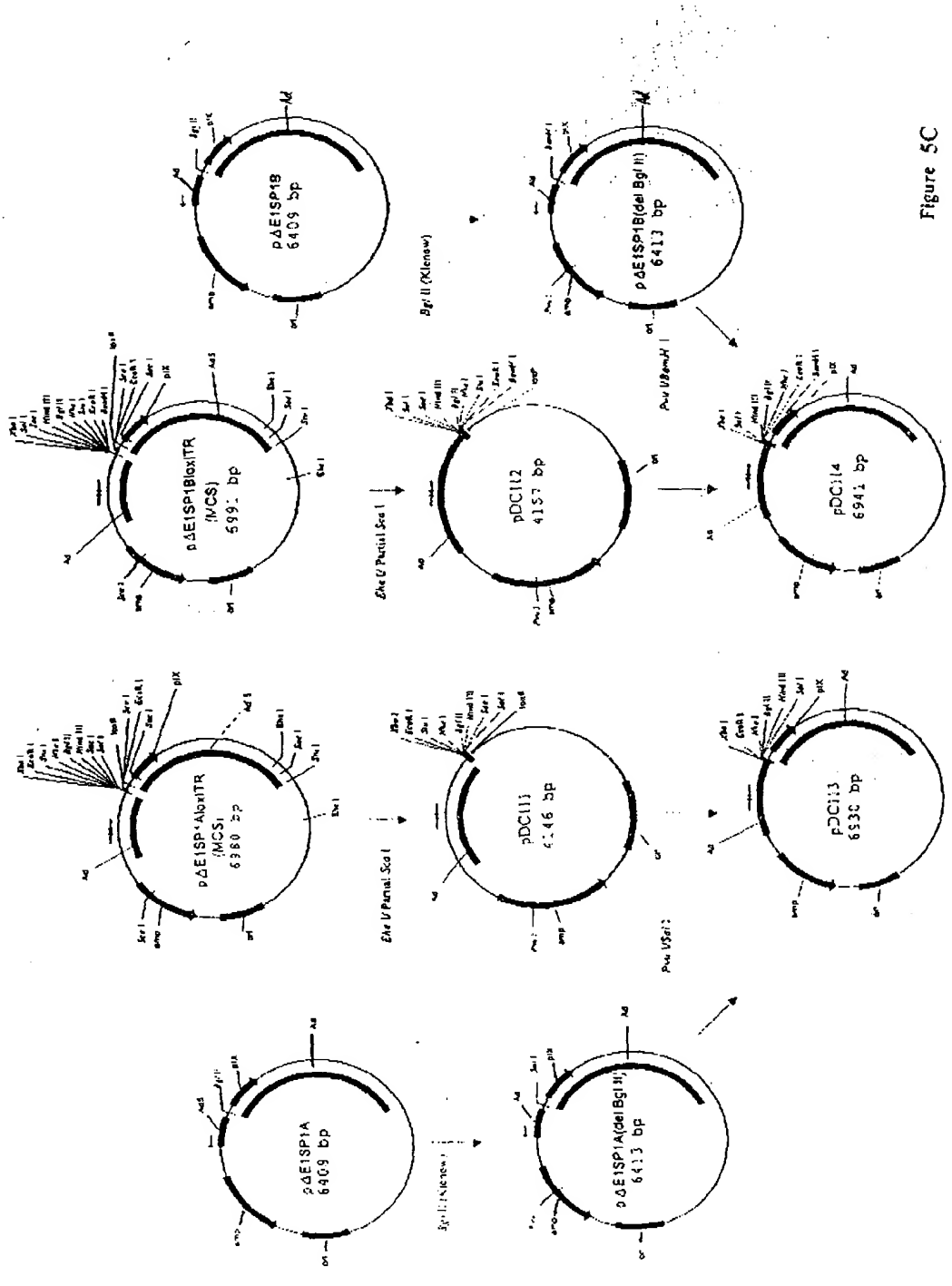
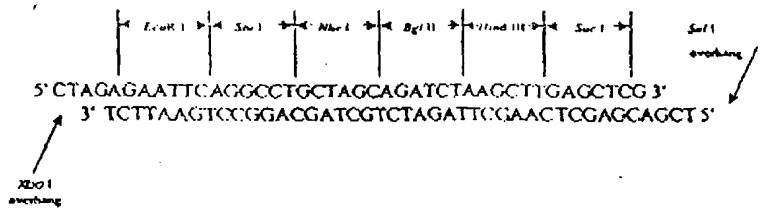



Figure 5C

Received from < 407 228 0329 > at 5/9/02 3:31:23 PM [Eastern Daylight Time]

SEQ. ID. NO.: 12 (AB16853) and SEQ. ID. NO.: 13 (AB16854)




SEQ. ID. NO.: 14 (AB16855) and SEQ. ID. NO.: 15 (AB16856)

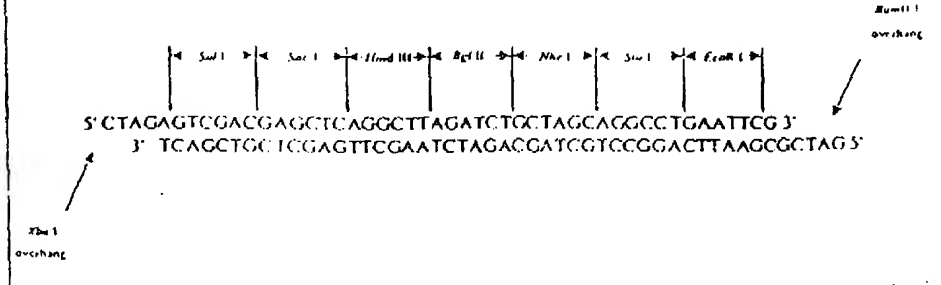



Figure 5D

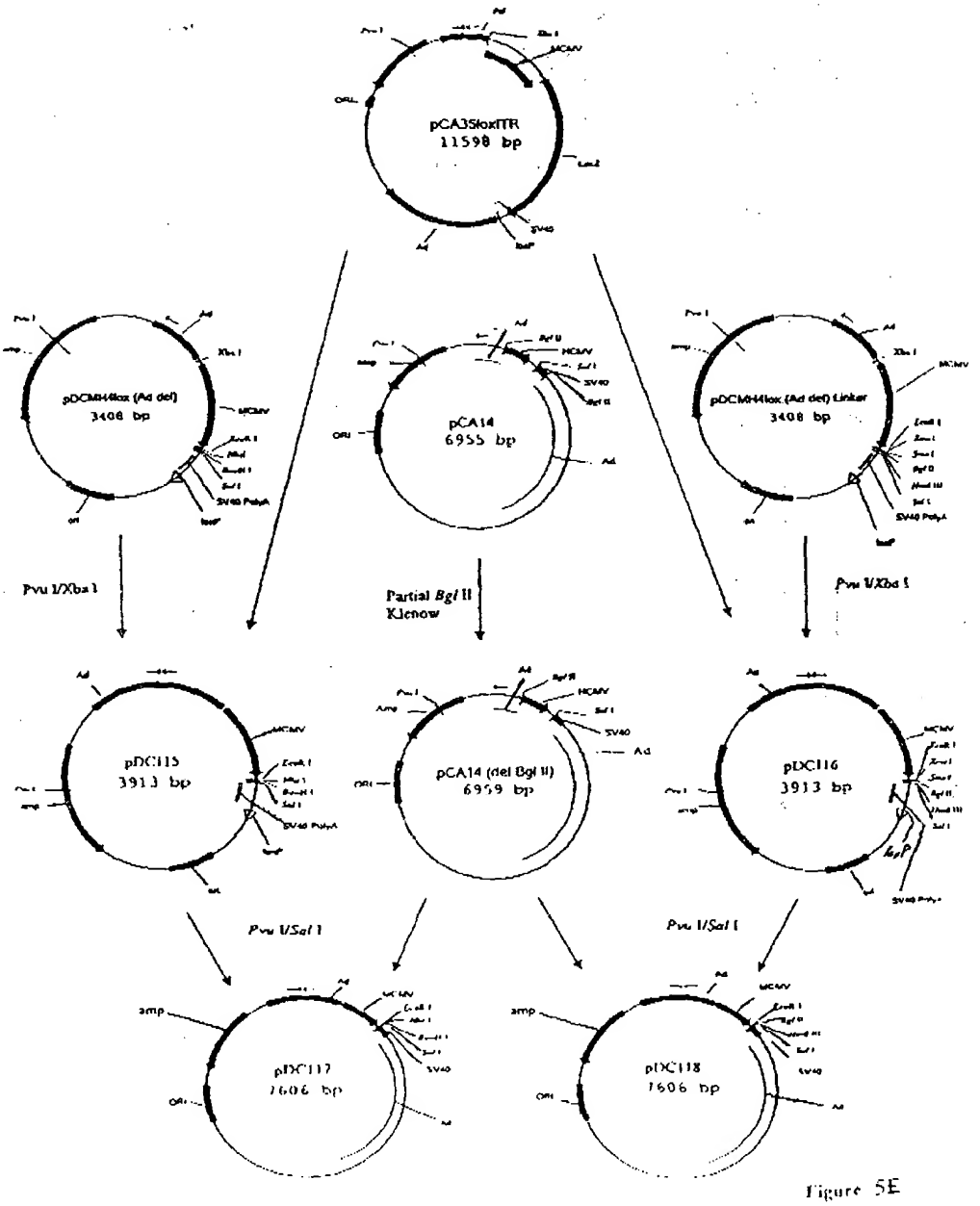

pCA3SloxITR
11598 bp
pDCMH4lox (Ad del)
3408 bp
pCA14
6955 bp
pDCMH4lox (Ad del) Linker
3408 bp
Pvu I/Xba I
Partial Bgl II
Klenow
Pvu I/Xba I
pDC115
3913 bp
pCA14 (del Bgl II)
6959 bp
pDC116
3913 bp
Pvu I/Sal I
Pvu I/Sal I
pDC117
7606 bp
pDC118
7606 bp

Figure 5E

# CONSTRUCTION OF pMH4LOX, pMH4LOXΔ and pMH4LOXΔLINK SHUTTLE PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

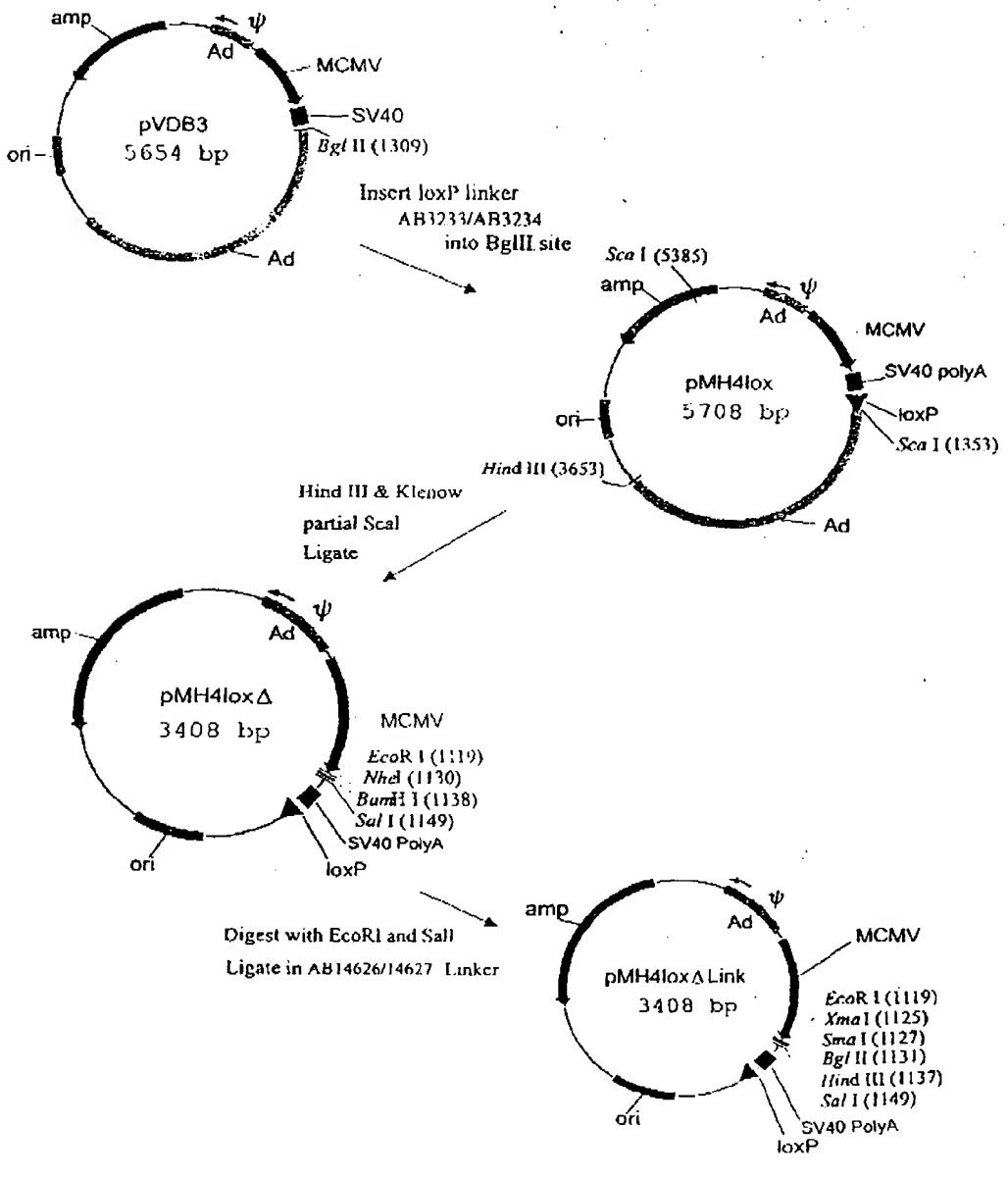



Fig. 6A

Received from < 407 228 0329 > at 5/9/02 3:31:23 PM [Eastern Daylight Time]

# CONSTRUCTION OF A SHUTTLE PLASMID CONTAINING A pUC DERIVED ORIGIN

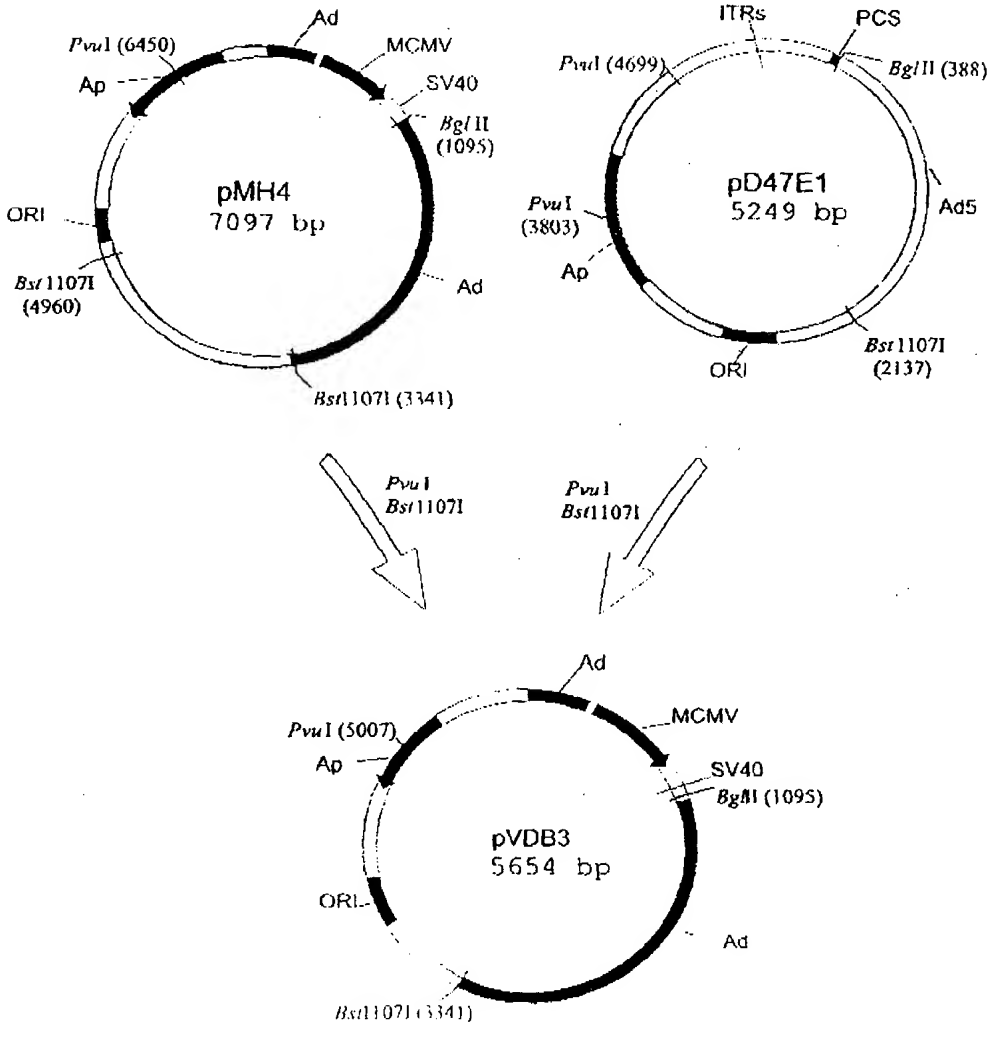



Fig. 6B

Received from < 407 228 0329 > at 5/9/02 3:31:23 PM [Eastern Daylight Time]

# CONSTRUCTION OF HCMV loxP PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

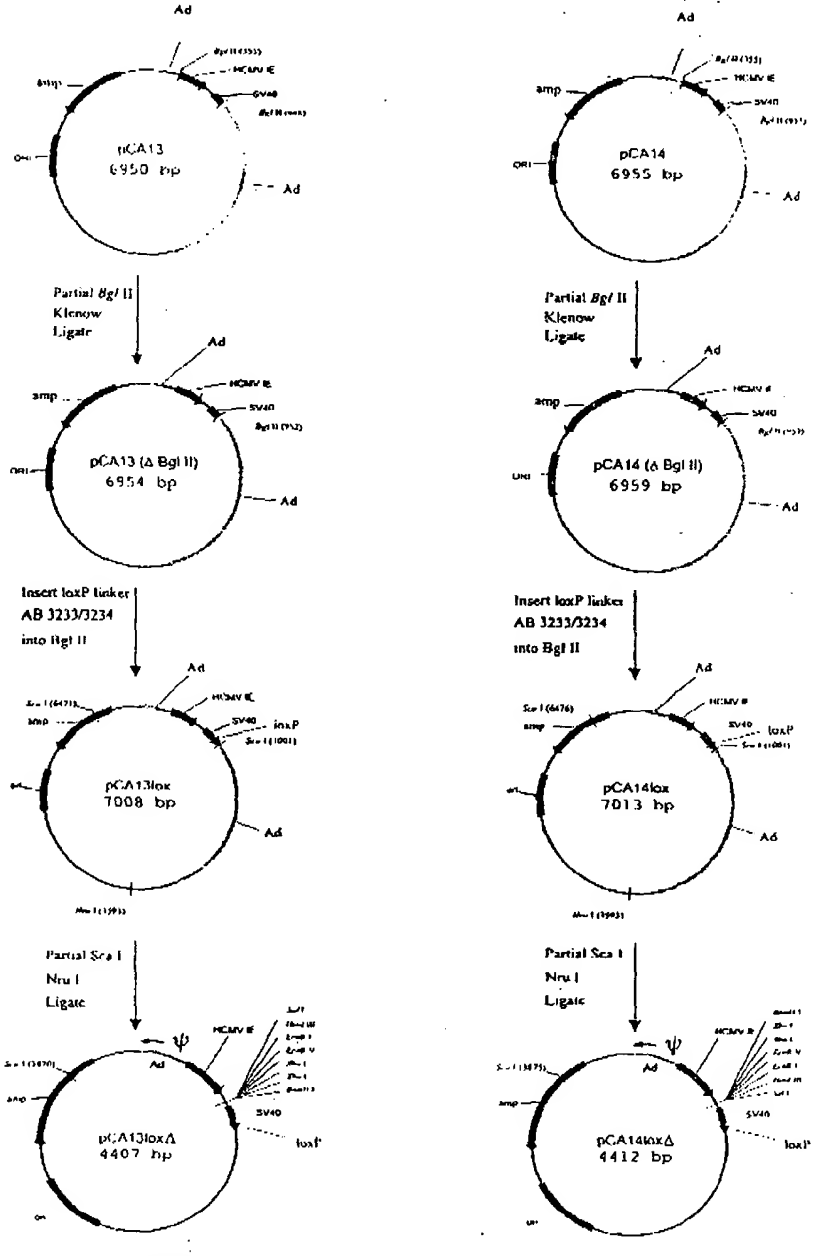



Fig. 7

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# CONSTRUCTION OF pCA36LOX and pCA36LOXΔ SHUTTLE PLASMIDS FOR RESCUE OF LACZ

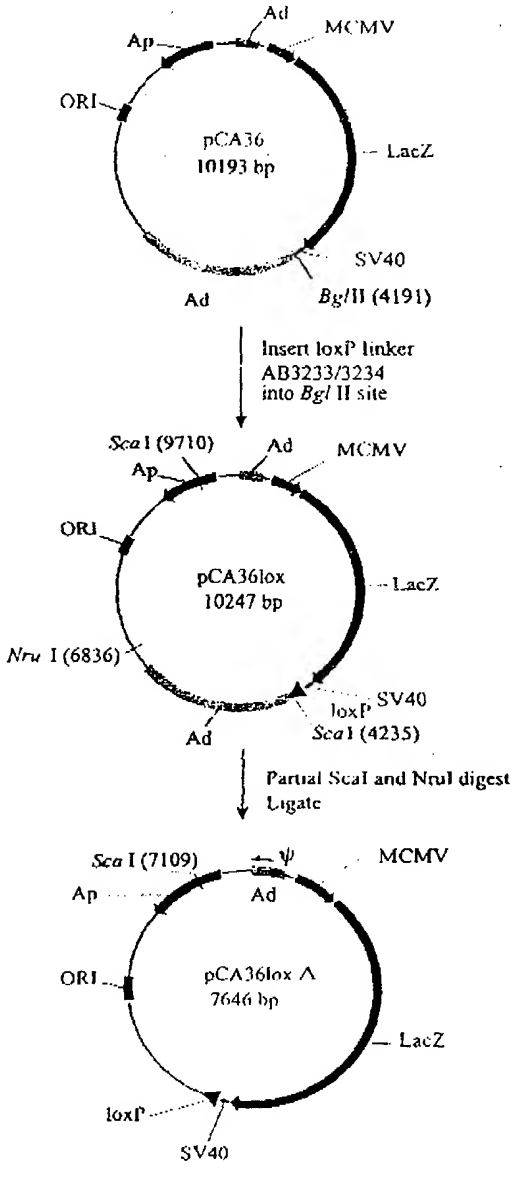


Fig. 8A

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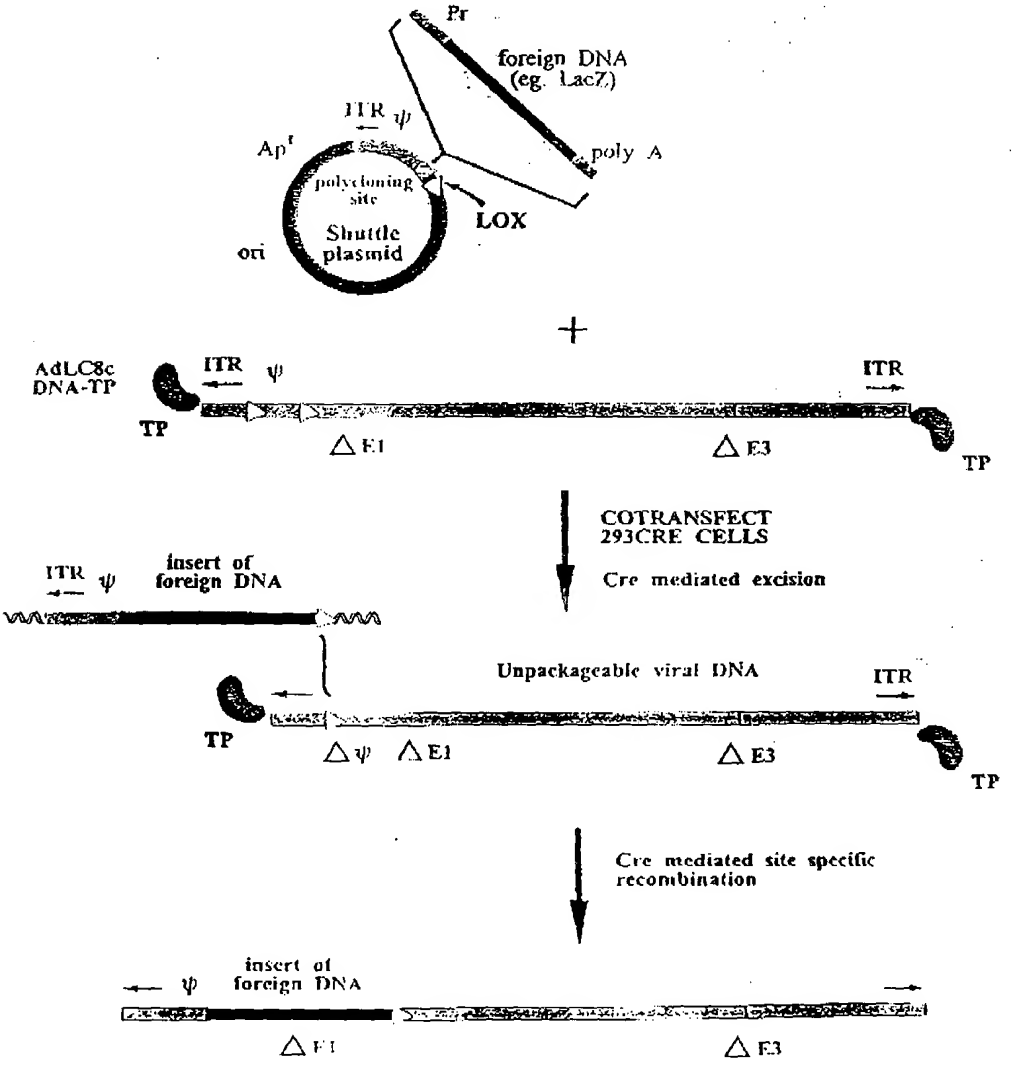
**Cotransfection of 293Cre cells with AdLC8c DNA-TP and a shuttle plasmid containing a loxP site for generation of Ad expression vectors**




DEFECTIVE VIRAL VECTOR

Fig. 8B

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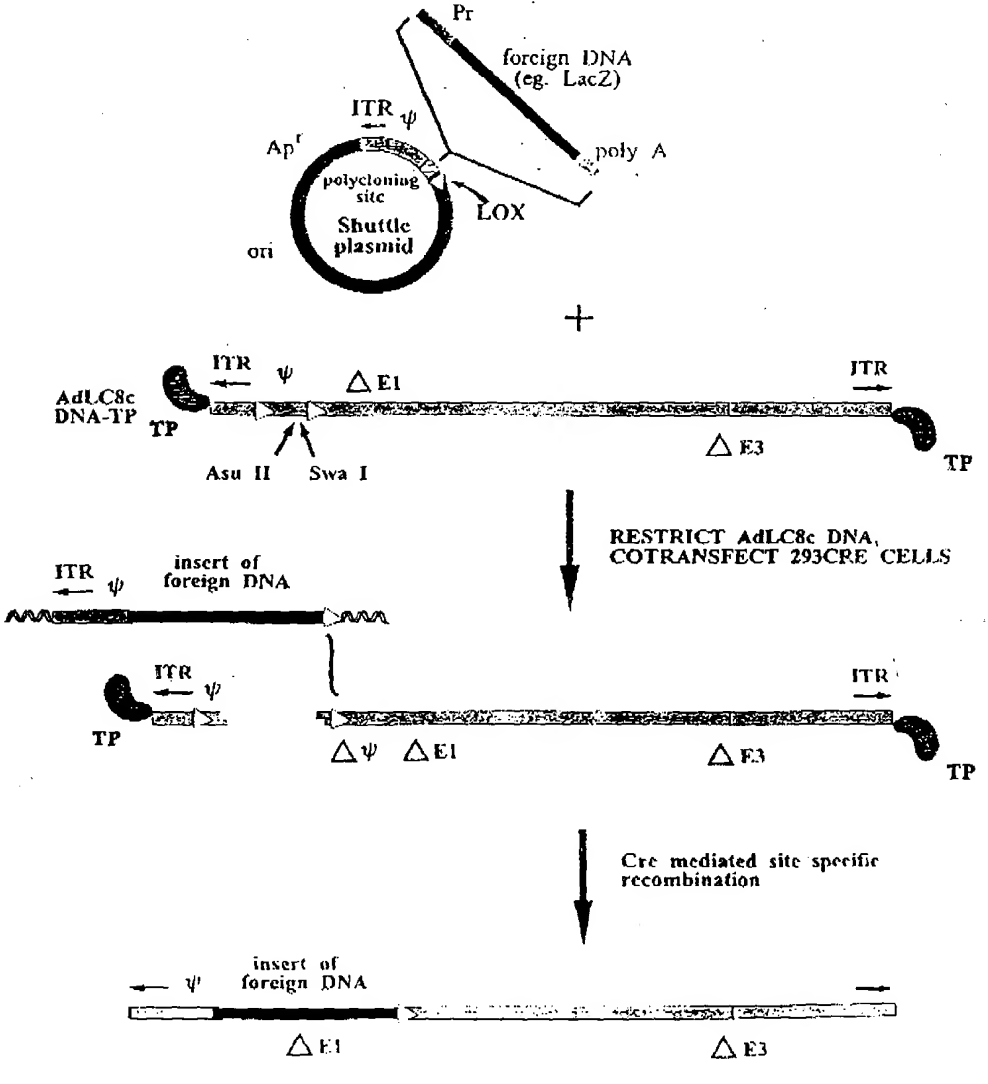
**Cotransfection of 293Cre cells with restricted AdLC8c DNA-TP and loxP shuttle plasmid for generation of Ad expression vectors**




DEFECTIVE VIRAL VECTOR

Fig. 8C

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CONSTRUCTION OF SHUTTLE PLASMIDS EXPRESSING Cre

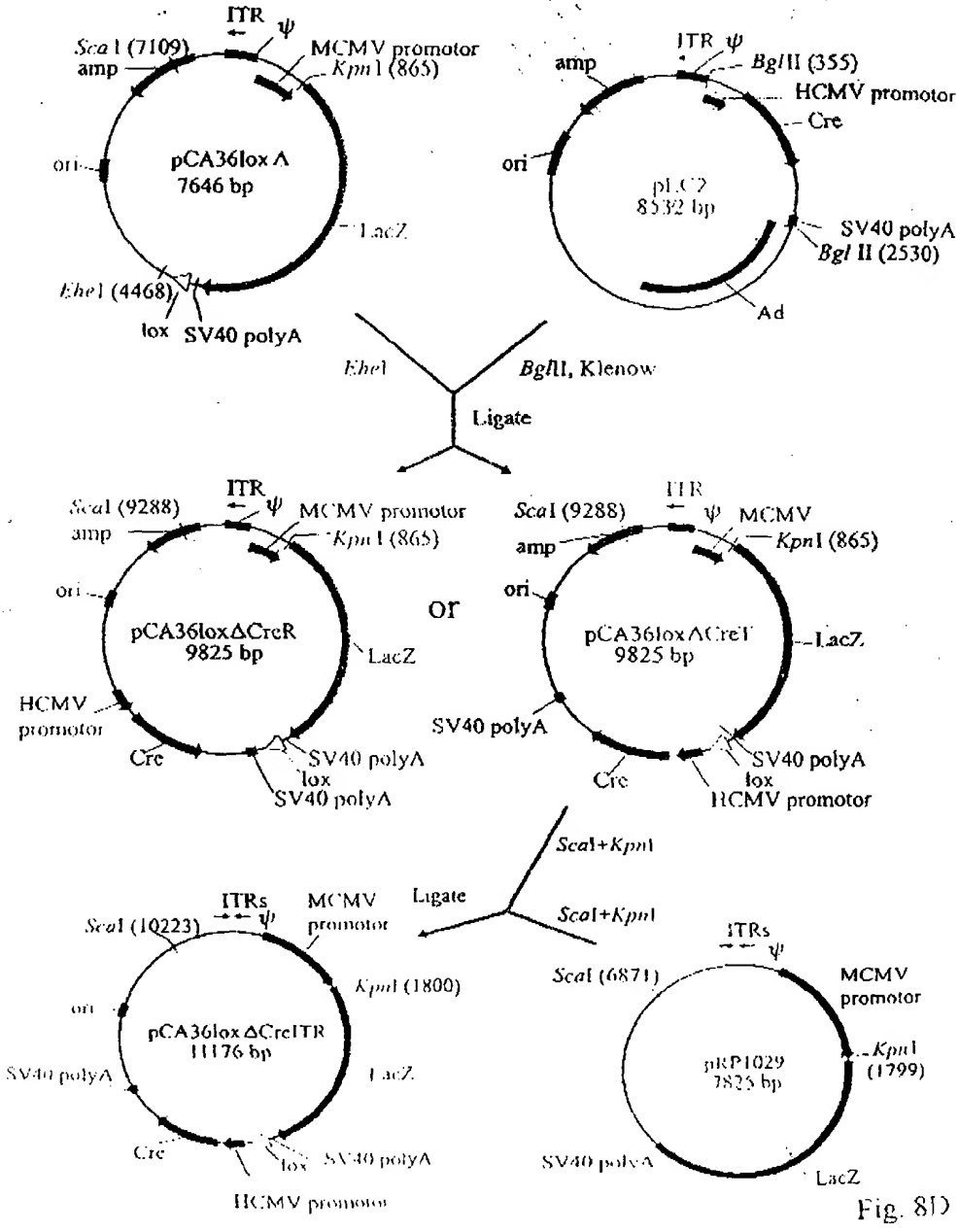


Fig. 8D

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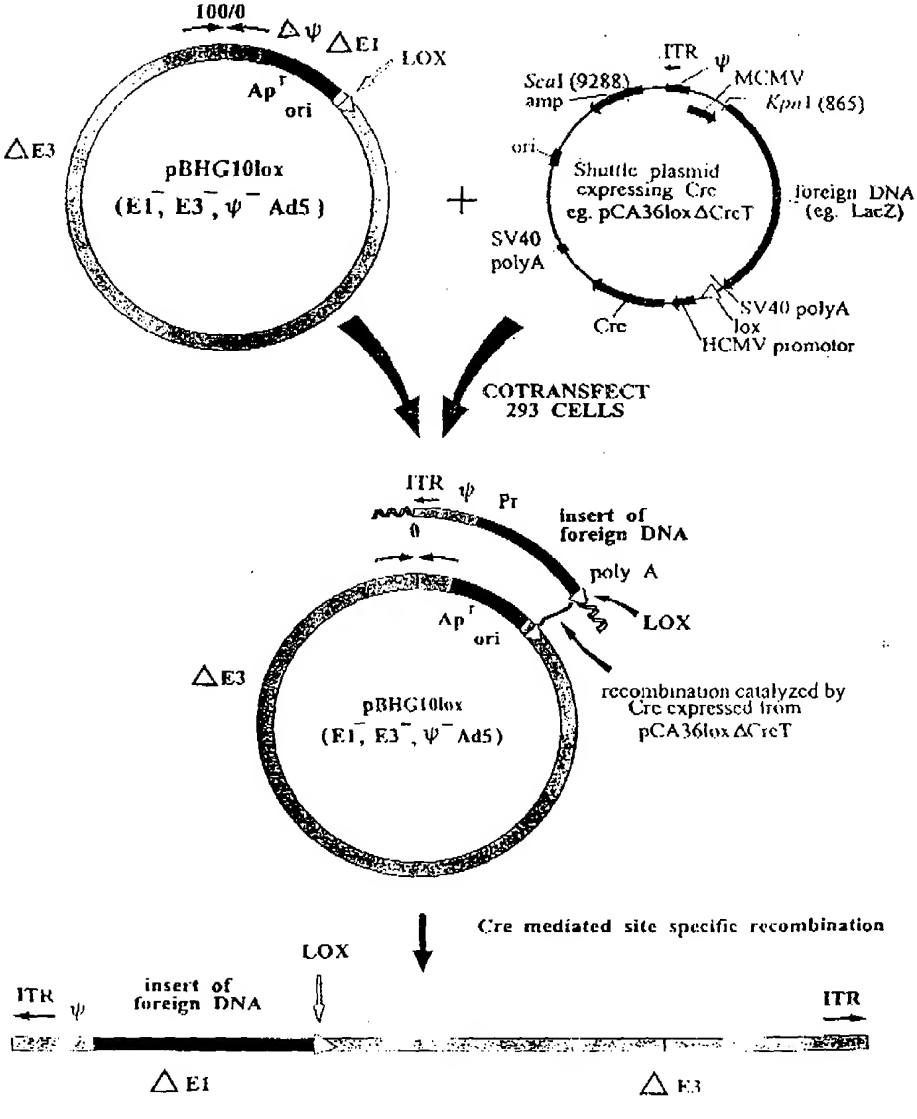
**Cotransfection of 293 cells with pBHG10lox and a "Lox" shuttle plasmid expressing Cre for generation of Ad expression vectors**




DEFECTIVE VIRAL VECTOR

Fig. 8E

Received from < 407 228 0329 > at 5/9/02 3:31:23 PM [Eastern Daylight Time]

# CONSTRUCTION OF Ad GENOMIC PLASMID ENCODING CRE

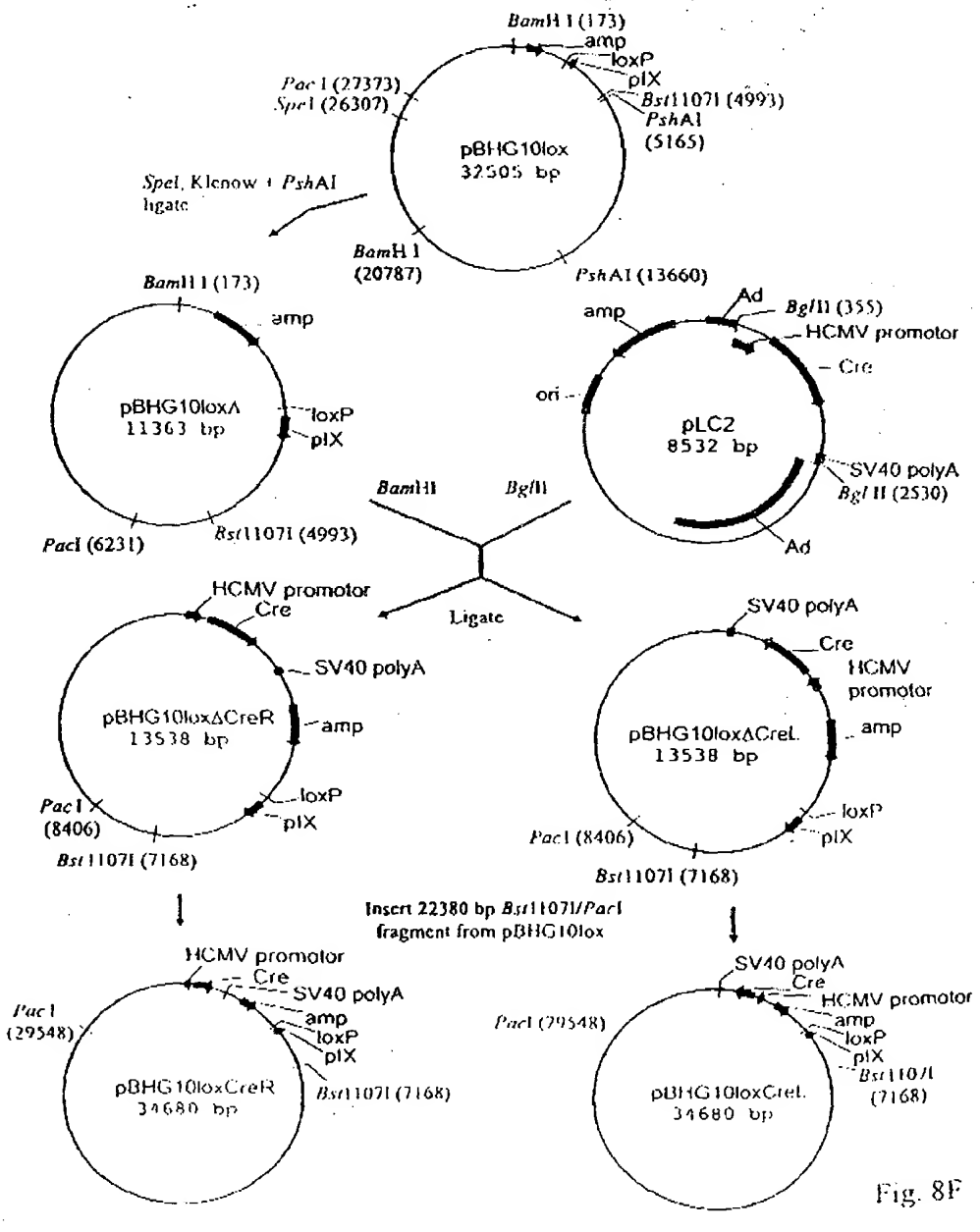



Fig. 8F

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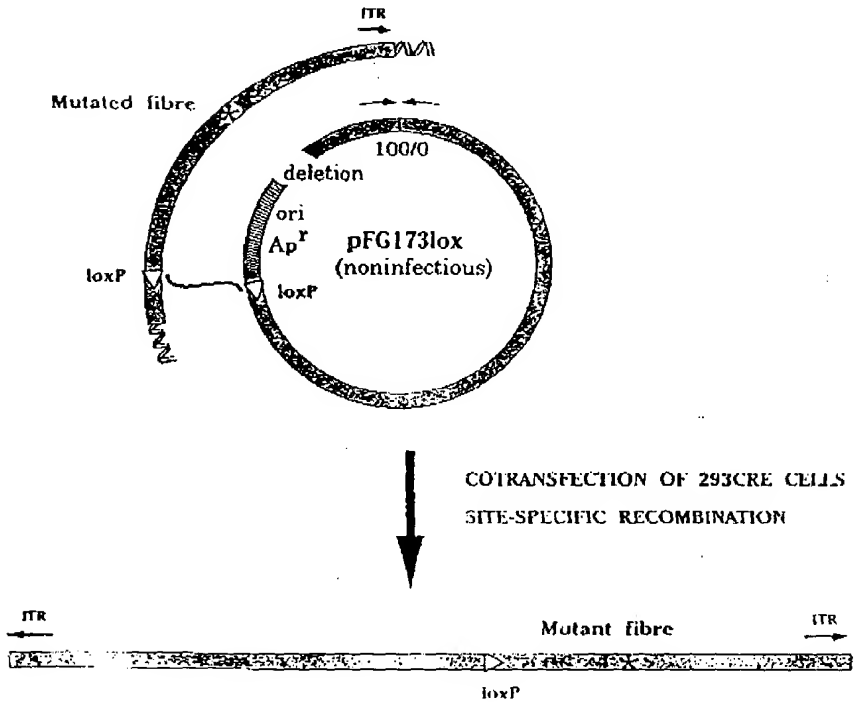
# RESCUE OF FIBRE MUTATIONS USING CRE/LOX RECOMBINATION




NONDEFECTIVE ($E1^+$) VIRUS WITH MUTATED FIBRE GENE

Fig. 9A

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# CONSTRUCTION OF pAB14lox △

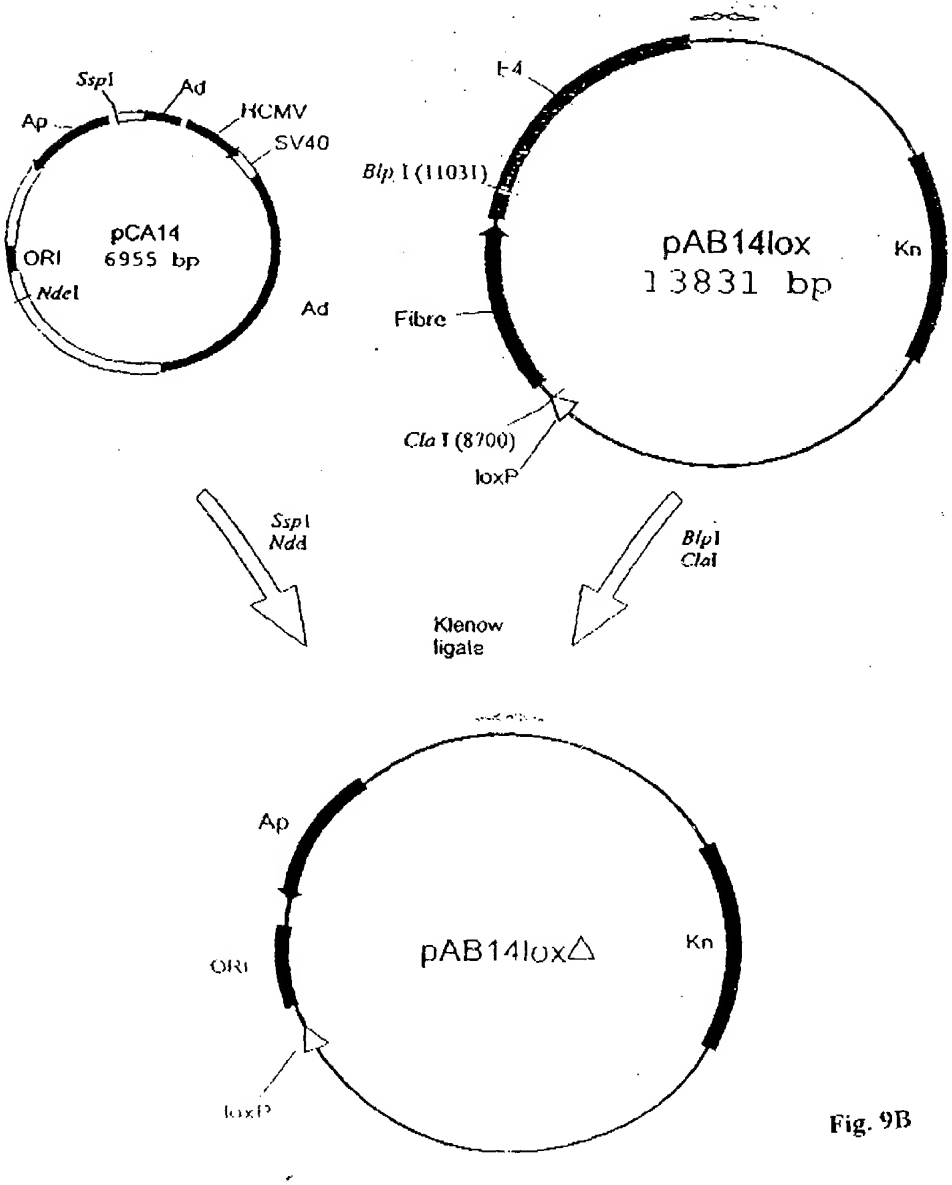
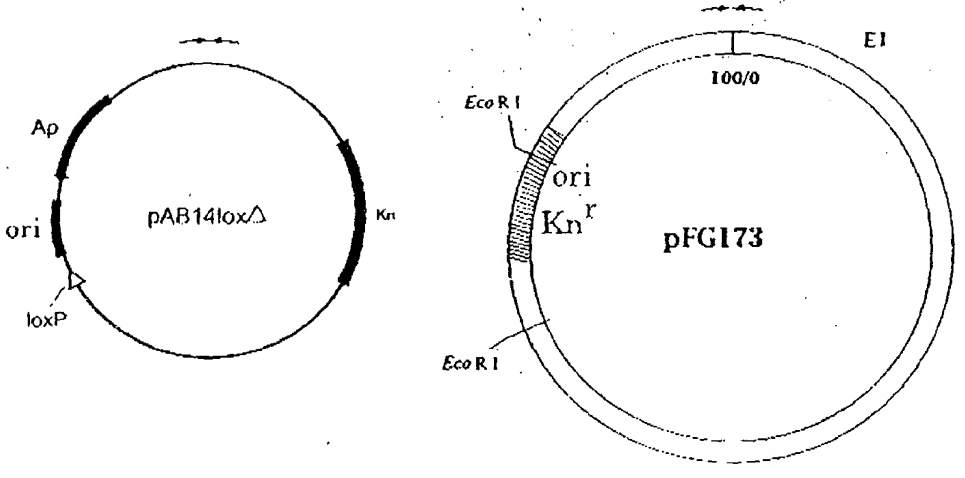



Fig. 9B

# CONSTRUCTION OF pFG173lox




**Restriction, transformation of E. coli, homologous recombination**

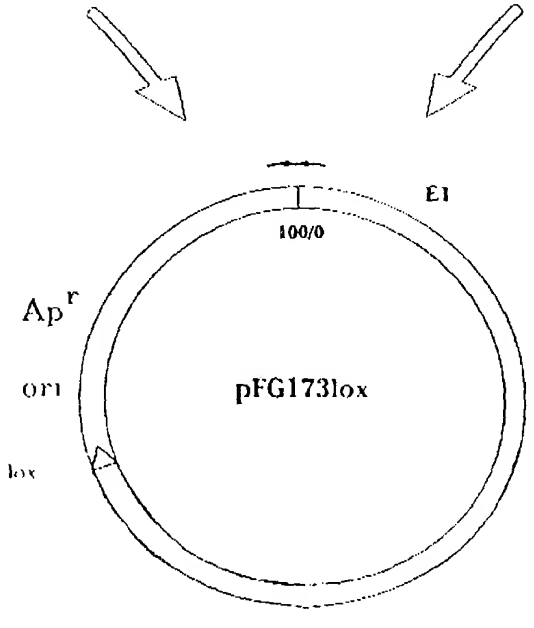



Fig. 9C

Received from <407 228 0329> at 5/9/02 3:31:23 PM [Eastern Daylight Time]

# CONSTRUCTION OF pFG23dX1lox AND pFG23dX1loxc FOR RESCUE OF MUTANT FIBRE INTO AD VIRUS

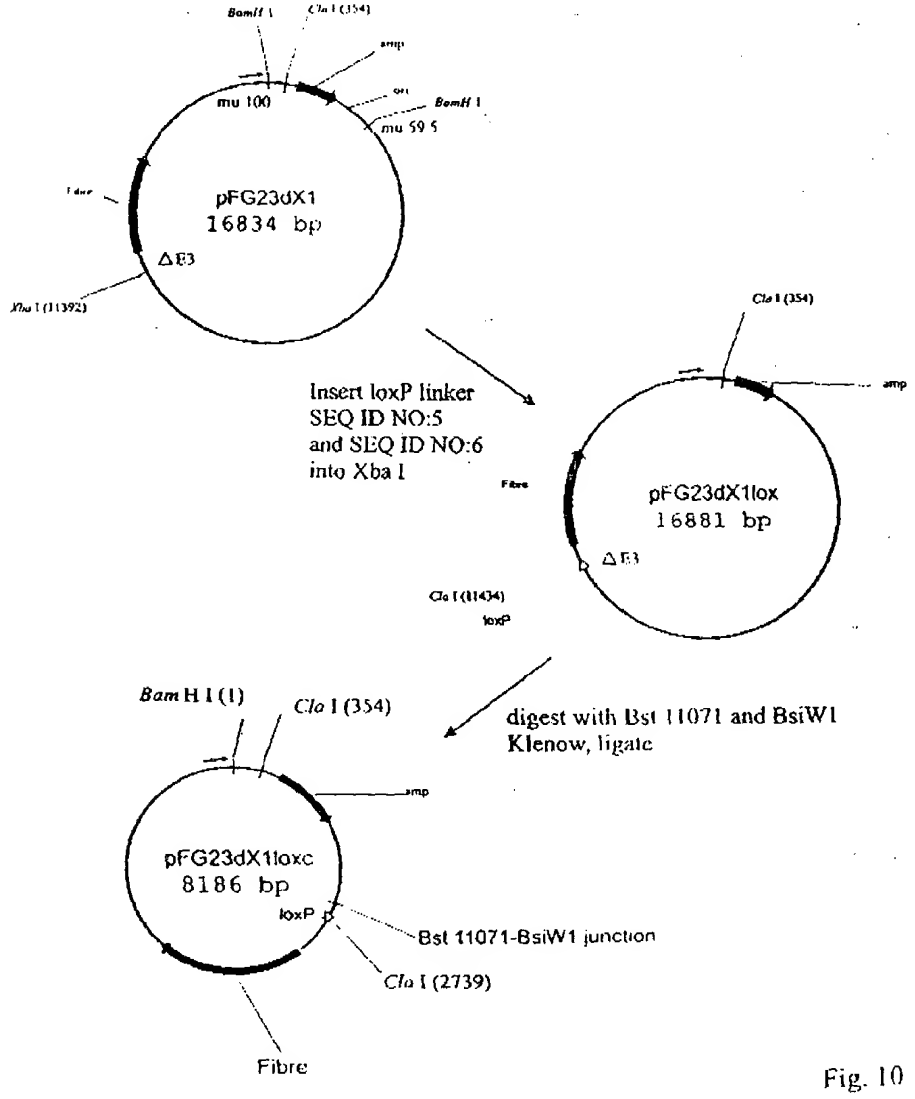



Fig. 10

# A PLASMID FOR RESCUE OF A FOREIGN DNA INTO AD VIRUS

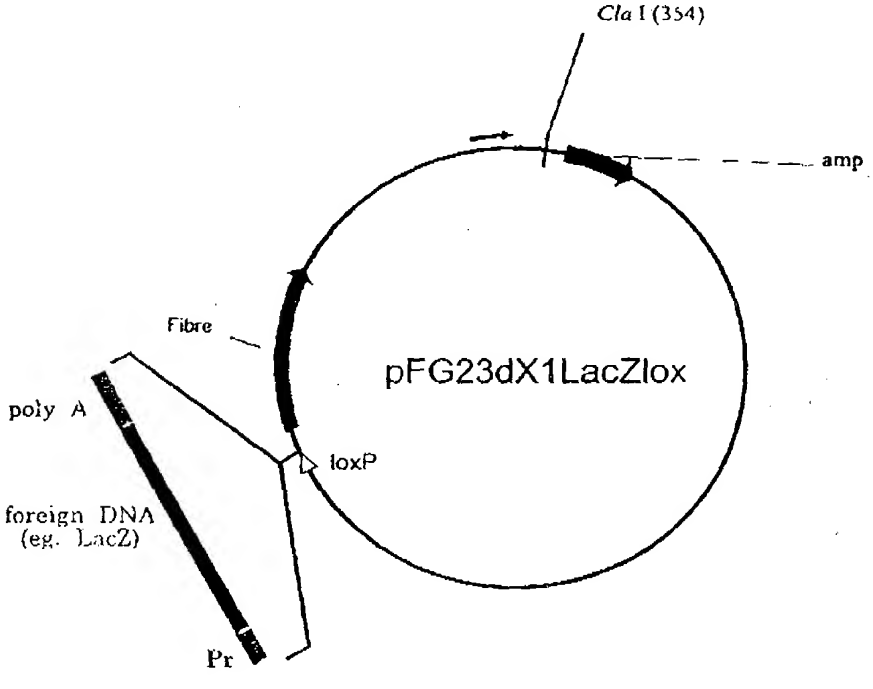



Fig. 11A

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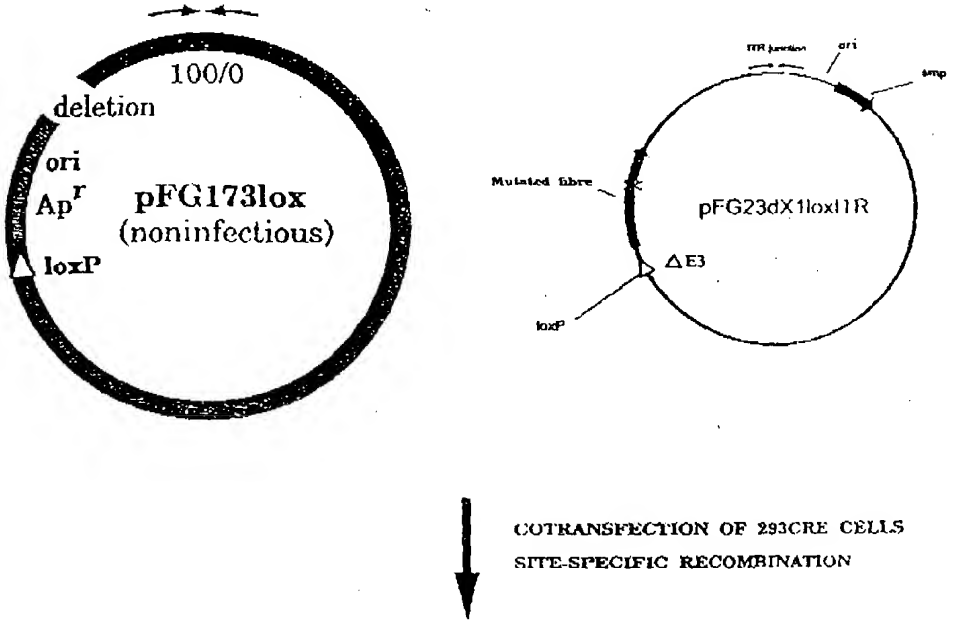
# RESCUE OF FIBRE MUTATIONS USING CRE/LOX RECOMBINATION




NONDEFECTIVE ($E1^+$) VIRUS WITH MUTATED FIBRE GENE

FIGURE 11B

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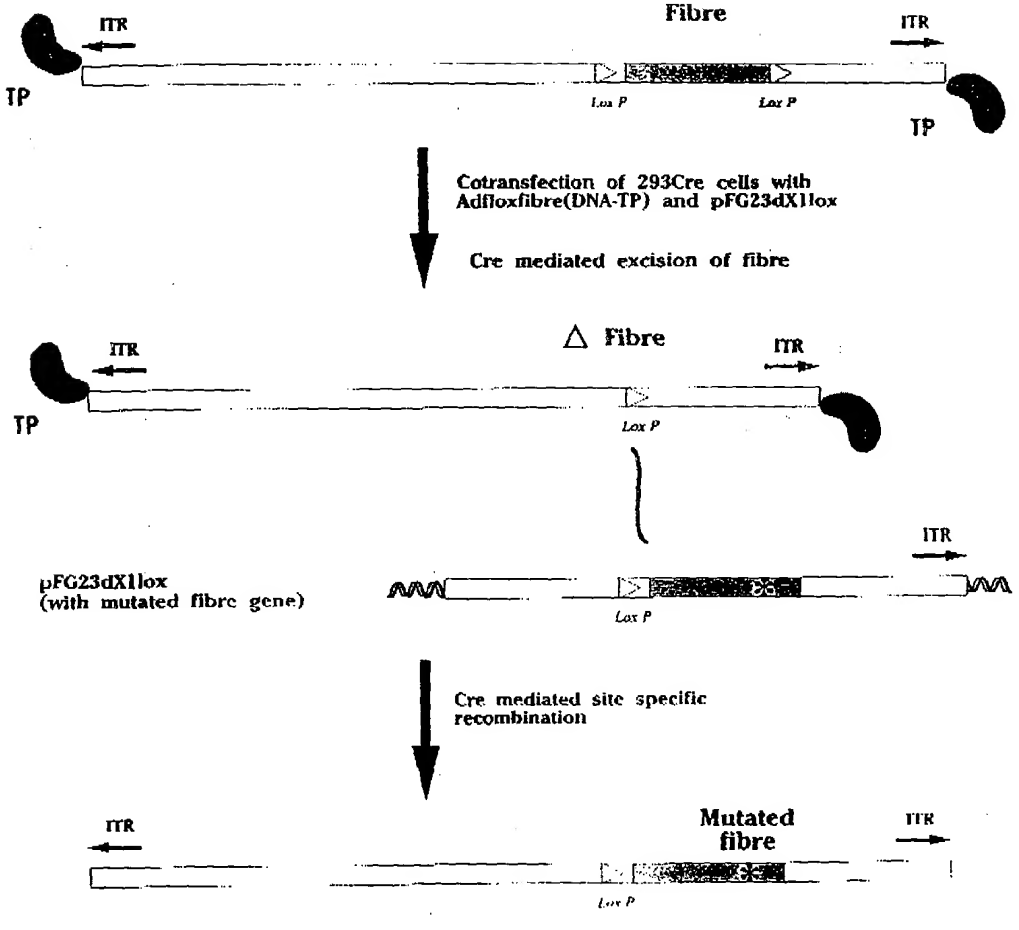
**Isolation of a virus containing a mutant fibre gene by Cre-lox recombination using DNA-TP and cotransfection**




RECOMBINANT VIRUS CONTAINING A MUTATED FIBRE GENE

Fig. 12

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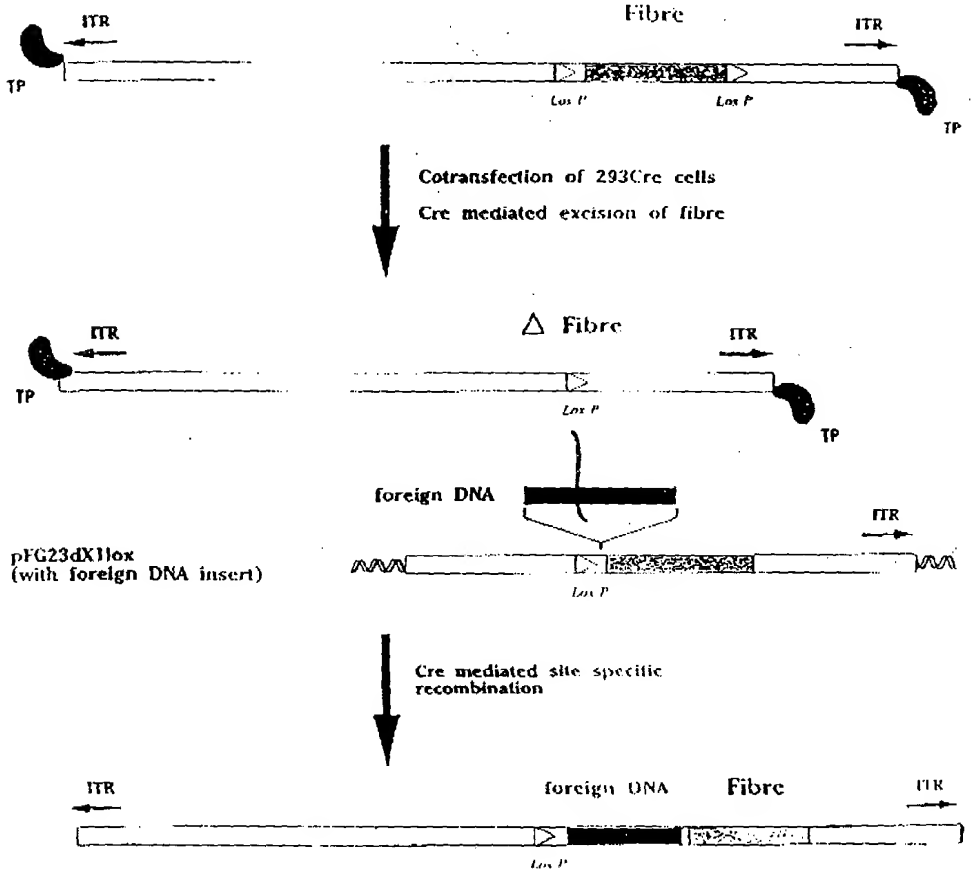
## Isolation of a virus containing a foreign DNA insert upstream of the fibre gene by Cre-lox recombination




RECOMBINANT VIRUS CONTAINING AN INSERT OF FOREIGN DNA UPSTREAM OF THE FIBRE GENE

Fig. 13

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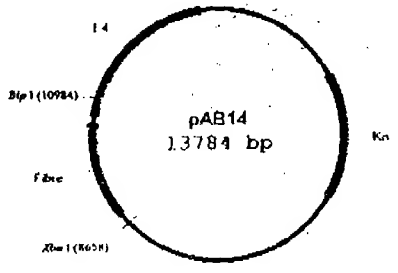
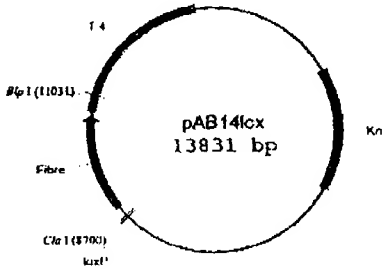
# CONSTRUCTION OF pAB14FLOX FOR ISOLATION OF AN AD VIRUS WITH A FLOXED FIBRE GENE




Insert loxP linker
AB6920/AB6921
into Xba I site




Insert loxP linker
AB14680/AB14681
into Blp I site

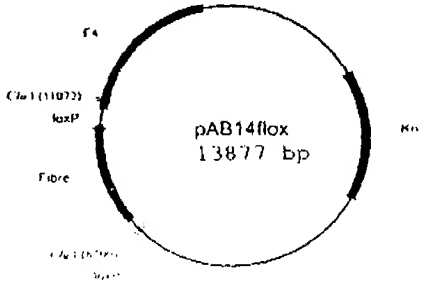



Fig. 14

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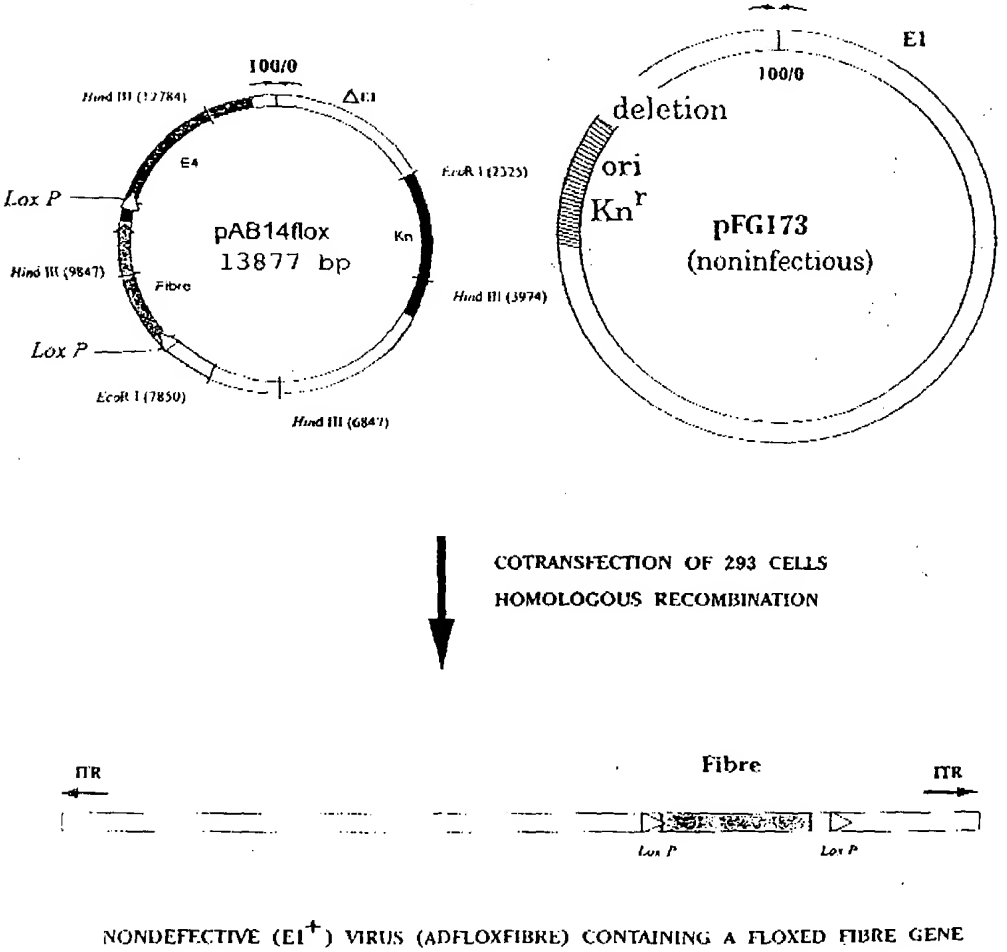
# Isolation of a virus containing a fibre gene with flanking lox P sites.




NONDEFECTIVE ($E1^+$) VIRUS (ADFLOXFIBRE) CONTAINING A FLOXED FIBRE GENE

Fig.15

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